BIOCHEMICAL PROFILES IN STRESS AND CANCER

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CERTIFICATE

I certify that the work presented in this thesis has been carried out by **Moayad Hosean Khataibeh** under my supervision. It is original in nature and has not been submitted for any other degree.

(Naheed Banu)
Reader

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Moayad H. Khataibeh

(Moayad H. Khataibeh)

Dedicated

to

My Parents

Contents

LIST OF ABBREVIATIONS

ACh	-	Acetylcholine
AChE	-	Acetylcholinestrase
ACTH	-	Adrenocorticotrophin hormone
BSA	-	Bovine serum albumin
CDNB	-	1-Chloro-2,4-dinitrobenzene
CRF	-	Corticotropin releasing factor
DADS	-	Dially disulfide
DATS	-	Diallyl trisulfide
DBH	-	Dopamine β-hydroxylase
DMBA	-	7,12 - Dimethylbenz (a) anthracene
DNA	-	Deoxy ribonucleic acid
DNPH	-	Dinitrophenyl hydrazine
DTNB	-	5,5'- dithiobis-2-nitrobenzoic acid
EDTA	-	Ethylenediaminetetraacetic acid
FRS	-	Free radical system
5-HT	-	5-Hydroxytryptamine (Serotonin)
gm	-	Gram
GOT	-	Glutamate oxaloacetate transaminases
GPT	-	Glutamate pyruvate transaminase
GSH	-	Reduced glutathione

GST	-	Glutathione-S-transferase
hr	-	Hour
HVA	-	Hydroxy vanillic acid
Kg	-	Kilogram
LDH	-	Lactate dehydrogenase
M	-	Molar
MAO	-	Monoamine oxidase
mg	-	Milligram
Min	-	Minute
µl	-	Microlitre
ml	-	Millilitre
µM	-	Micromolar
N	-	Normal
NADH	-	Nicotine adenine dinucleotide hydride
nm	-	Nanometer
nM	-	Nanomole
OD	-	Optical density
PBS	-	Phosphate buffered saline
PCA	-	Perchloric acid
PNMT	-	Phenylethanolamine-N-methyl transferase
PU	-	Provisional Unit
RBC	-	Red blood Cell

SAC	-	S-allayl cysteine
SD	-	Spraque - Dawley
SEM	-	Standard error of mean
SOD	-	Superoxide dismutase
TCA	-	Trichloro acetic acid
TEB	-	Terminal end buds
Tris	-	Tris (hydroxymethyl) aminomethane
VMA	-	Venyl mandelic acid
W/V	-	Weight/Volume

Abbreviation for statistical p values in Table

a, a', a"	-	< 0.001
b, b', b"	-	< 0.01
c, c', c"	-	< 0.05
d, d', d"	-	< 0.1

LIST OF CHEMICALS

Acetone	-	Qualigens
Acetylthiocholineiodide	-	Sigma Chem. Co.
α-Ketoglutaric acid		SRL
Aspartic acid	-	SRL
Benzylamine	-	E.Merk
BSA	-	SRL
CDNB	-	Sigma Chem. Co.
Pyrogallol	-	Qualigens
Cortisol	-	Sigma Chem. Co.
Cyclohexane	-	Qualigens
Dichloromethane	-	Qualigens
Disodium hydrogen ortho-phosphate dihydrate	-	Qualigens
Sodium hydrogen phosphate -2-hydrate cryst Pure	-	E. Merk
Potassium sodium tartarate	-	Qualigens
Sodium carbonate	-	Qualigens
Sodium hydroxide	-	Qualigens
Pyruvate	-	SRL
Sodium hydroxide	-	qualigens
DMBA	-	Sigma Chem. Co.

DTNB	-	Sigma Chem. Co.
Succinic acid	-	BDH
EDTA	-	Ranbaxy Laboratries Limited,
Folin's	-	SRL
Glutathione	-	SRL
HCL	-	SRL
Heparin	-	Gland Pharma
H_2SO_4	-	Qualigens
KCL	-	Qualigenes
Tris	-	SRL
TCA	-	Qualigens
L-Alanine	-	SRL
NADH	-	SRL
Sodium pyruvate	-	SRL
Sodium chloride	-	Qualigens
Sodium pentobarbital	-	Abbott Laboratries

Introduction

The literal meaning of the word 'Stress' is 'Constraining force', while the language of Life-science, defines 'stress' as an intense force, strain, agent or mental condition producing a defence reaction, which if continued or intensified, may lead to pathological lesion. According to Selye (1956), stress is the non-specific response of the organism to any demand made upon it. Rabkin and Struening (1976) said that stress like anxiety, was a broad and general concept describing organism's reactions to environmental demands. Chronic stress in human can cause psychological and physiological reactions (Cholst, 1996). Selye (1976) described the sequence of pathological changes occuring in the animals following exposure to stressful stimuli. He named it as "General Adaptation Syndrome" (G.A.S.) which developes in three stages comprising of : i) Alarm reaction or shock. ii) Stage of resistance and iii) Stage of exhaustion. Alarm reaction consisted of the triad of lymphothymic involution gastrointestinal ulceration, and loss of cortical lipid and medullary chromaffin substance from the adrenals. If the effect of stressful stimuli continues for a long period, the body develops the state of resistance. However, in case the stress is sufficiently severe and prolonged and the body fails to adapt, it may lead to the state of exhaustion in which the animal developes symptoms similar to those seen in the first stage.

Endocrinal, neurohumoral and antioxidant scavenging system's response to stress :

The relationship between stress, hormones and various metabolizing enzymes is now well established. During stress increase in the activity of

sympatho-hypothalamo-pituitary-adrenal system has been observed (Kvetnansky and Mikulaji, 1970 and D'Amato, 1992). In response to stress, the tropic hormones which in turn by acting on their target endocrine glands stimulate the synthesis and release of their respective hormones (Levi, 1967). Thus, due to stress the circulating levels of catecholamines, cortisol, ACTH, growth hormones, acetylcholine, histamine have been found considerably enhanced (Glick *et al.*, 1965; Berson and yelow, 1968; Kvetnansky, 1972; Mikulaji *et al.*, 1975; Pandey, 1976; Rai, 1976; Kopin *et al.*, 1980 and Parrott, 1994).

An increased acetylcholine level is observed after stress (Aprison and Hington, 1969 and Parrott, 1994). The oscillation stress is found to deplete brain ACh levels and strain stress increases it in animals (Satio *et al.*, 1976). The activity of the catabolizing enzyme of ACh i.e. AChE is found increased in the students with increased body temperature during examination (Yardanova and Gotsa, 1971), while a significant decrease in its activity is observed in experimental animals under stress (Litvak, 1969). Gupta *et al.*, (1978) have reported an enhanced ACh level in psychic stress.

Increased levels of both ACh and AChE are reported after electric shock (Singh *et al.*, 1980). The blood levels of ACh were found significantly enhanced in the patients of diabetes mellitus (Kamysheva and Gasperovich, 1978), and after excercise (Basu *et al.*, 1975 and Weltman, 1994).

The metabolism of catecholamines have also been found altered in response to stressful stimuli (Subramanium, 1973; Scheider *et al.*, 1974 and

Mason, *et al.*, 1976). In various stressful conditions the activities of catecholamine synthesizing enzymes have been observed to be enhanced (Weinshilboum, *et al.*, 1971; Kvetnansky, *et al.*, 1976 and Rysanek, *et al.*, 1978). Increased dopamine -b-hydroxylase activity with a decreased MAO activity has been recorded following stimulation of sympathetic nervous system or exposure to stress (Weinshilboum *et al.*, 1971 and Sharma, 1978). However, hypophysectomized animals failed to exhibit such changes in DBH activity following exposure to stress (Molinoff, 1970) . The activity of MAO is decreased in hypophysectomized animals or after hydrocroticosterone treatment (Parvez and Parvez, 1973), while an increase in brain MAO activity is found after adrenalectomy (Ceasar *et al.*, 1970). MAO activity was found to be decreased significantly (Both A and B) in immobilization stress (Obata and Yamanaka, 1994). Guelman (1996) reported the change in the MAO (A & B) in adult rat cerebellum following neonatal X-irradiation.

Barchas and Freedman (1963) has reported that physiological stress modifies the serotonergic activity with an alteration in its metabolising enzymes. Several other workers have also reported the action of stress on central nervous system (Bliss *et al.*, 1968; Bliss, 1973 and Modigh, 1974). 5-HT metabolism is found to be accelerated in the central nervous system by various stressors (Bliss *et al.*, 1968; Bliss, 1973; Bourgoin *et al.*, 1973 and Thierry, 1973). The plasma and urinary 5-HT levels have been found increased after exposure to a variety of stress such as cold stress, immobilization stress, electric shock etc. (Toh, 1960; Sarkar, 1978 and

Hirvonan *et al.*, 1978), while some workers have reported a decrease in 5-HT level after stress (Corrodi *et al.*, 1968). The involvement of stress associated with certain disease conditions is well known; shch as in Schizophrenia (Smythies, 1976), Peptic Ulcer (Udupa, 1978), where central 5-HT metabolism is disturbed with the association of decreased platelet monoamine oxidase (Wyatt *et al.*, 1973 and Domino *et al.*, 1976).

The stress induced hyperactivity of adrenal cortex was originally described by Selye (1946). According to Henry (1977), the psychosocial stress activates either the pituitary-adreno-cortical system or the sympatho-adreno-medullary system. The adrenocortical system becomes activated when the organism fails to compete with the situation, a state leading to depression. In this type of stress the ACTH and corticosteroid levels are augmented with unaltered catecholamine levels. During the fight-flight reaction i.e. the organism fears a challenge to its integrity and maintenance of homeostasis, there occurs an increase in the sympatho-adrenomedullary system. This type of stress is characterized by an increased level of noradrenaline and adrenaline, while corticosterone remains unchanged. An increase in circulating and urinary levels of corticosteroids is found following various stressful stimuli, as surgical trauma, pain, anaesthesia and psychic stress (Thomasson, 1959 and Hume *et al.*, 1962). Enhanced levels of cortico-steroids have also been reported in a variety of diseased conditions (Schimkin, 1943 and Lovegrovement *et al.*, 1965) and experimental studies related to stressful conditions (Von Euler, 1969; Kvetnansky, 1972; Mikulaji *et al.*, 1975 and

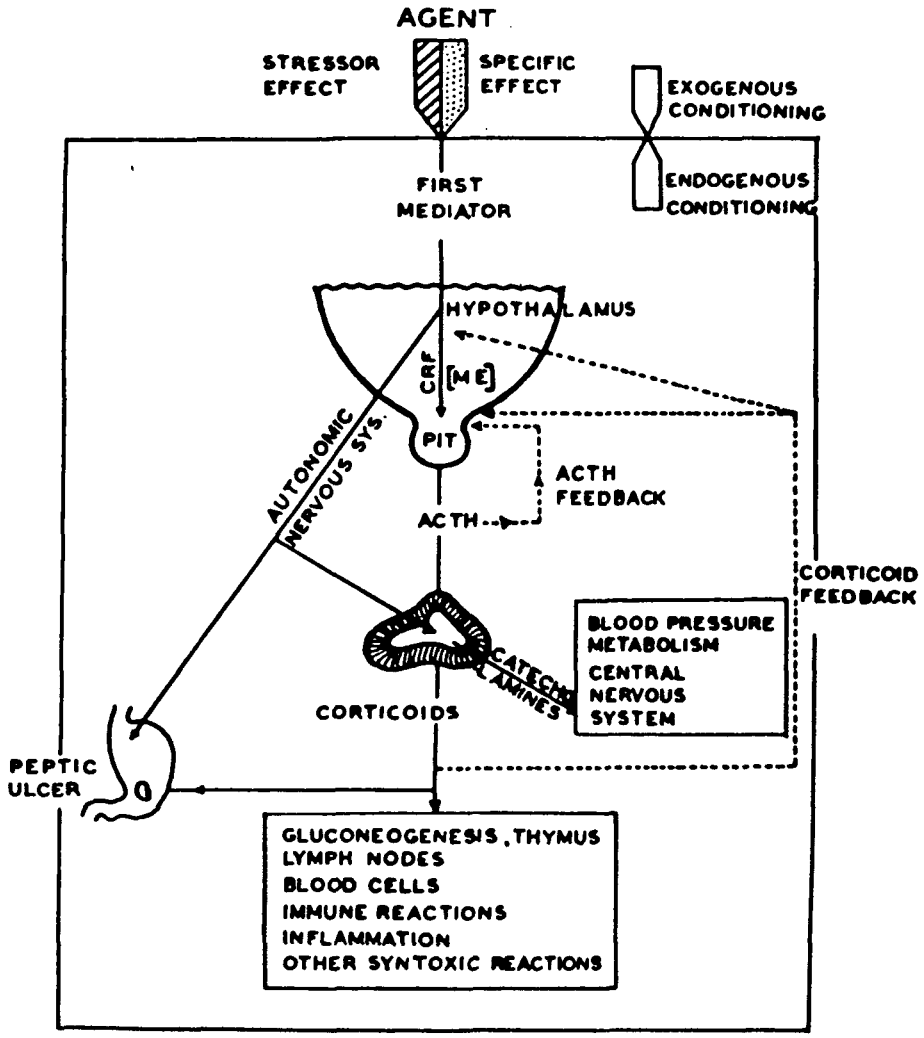


Fig. 1

Principal pathways mediating the response to any stressor agents and conditioning factors which modify its effect (courtesy Sely, 1976). (Butter worths Boston and London)

Szentendrci *et al.*, 1980). In such situations the stress response is characterized predominantly by pituitary adreno-cortical system rather than the sympathetic aderenolmedullary system, as described by Mason (1975). During depression, pituitary control of the adrenal cortex is affected with a consequent elevation of ACTH and plasma cortisol (Corroll, 1976 and Levine *et al.*, 1978).

Glutathione as biological antioxidant, plays a role in the destruction of free radicals, (Sohal *et al.*, 1984), and cellular protection during aging (Sohal *et al.*, 1984 and Pruche *et al.*, 1991). Enhanced level of lipid peroxides along with depletion of glutathione has been observed during stress (Younes and Siegers, 1980 and Katoh *et al.*, 1989). Depletion of glutathione during immobilization stress is reported to stimulate oxidants and oxidative damage contributing to the degenerative diseases of ageing including brain dysfunction (Lin *et al.*, 1996). Glutathione is essential for the repair process in brain exposed to oxidative damage by free radicals (Pellmur *et al.*, 1992).

Considerable interest has been directed towards concentrations of glutathione in brain and other tissues mainly because this tripeptide is considered to have important functions in protecting cells against oxidative damage (Orlowski and Karkowsky, 1976). In this capacity, reduced glutathione (GSH) can act as a free radical scavenger (Rink, 1974 and Jaroslava *et al.*, 1979). It has been reported that mild hypoxia in rats reduces brain tissue concentrations of GSH (Wideman and Domanska, 1974 and Jaroslava *et al.*, 1979). Effect of antioxidant and free radical scavenging systems exist in the

cell protection against the damage resulted by free radical produced as a part of normal cell respiration and other cellular processes such as inflammatory response (Flohe *et al.*, 1973; Willson, 1980, 1983; Cohen, 1984; Tappel, 1984 and Kaplotiwitz *et al.*, 1985). The involvement of free radical and free radical reactions have been observed in the etiology and development of a number of diseases, especially life limiting (Pryor, 1978). Role of reactive oxygen species have been reported in oxidative stress related diseases (Sies, 1991). Oxidative damage to lipids, protein and DNA in the brain has been observed during immobilization stress (Liu *et al.*, 1996). Immobilization stress induces generation of reactive oxygen species and decreases the endogenous antioxidants defenses, which can be attenuated by extra cellular adminstration of antioxidant GSH. (Liu *et al.*, 1994).

The enzyme superoxide dismutase has been demonstrated in a variety of tissues and cell types and appears to protect against the toxic effects of oxygen free radical and thus provides a mechanism whereby an organism can protect possible deleterious effects of this radical or other free radicals produced by its further reaction with cellular components (Fridovich, 1975 and McCord *et al.*, 1971). SOD have been reported as the first enzyme of the scavenging enzyme series to controlled the damage caused in cells by free radical (Slater, 1984). Singlet oxygen and superoxide radical have been observed potentially toxic to living cells as they can participate in the oxidation of cell macromolecules like protein, lipids etc. in case of leakage from the original oxidation reactions (King *et al.*, 1975).

Generation of superoxide anion during interaction of molecular oxygen with flavins, NADH, glutathione peroxidase and catecholamines has also been studied (Misra and Fridovich, 1972). It has been observed that immobilization stress induces antioxidant defense changes in the plasma of rats (Liu *et al.*, 1994). Several workers have reported the role of oxygen free radical and the role of SOD in clinical study on stress gastritis prophylaxis (Kayabali *et al.*, 1994 and Zhang, 1993). Decrease in the activity of SOD has been observed in peptic ulcer and in the patients with lesions of the hepatobiliary system along with depletion of glutathione (Kolomoets, 1992), whereas, no significant changes in the activity of glutathione-S-transferase was observed. It has been reported that GST has a major role in the detoxification of oxyradicals and their products (Mannervik and Danielson, 1988). Brain GST plays an important role in the detoxification of potential toxicants through their conjugation and biotransformation (Booth *et al.*, 1961; Boyland and Chasseand., 1969; Dixit *et al.*, 1980 and Kuboto *et al.*, 1985). Greater accumulation of the toxic compound is reported to inhibit the GST activity (Boyland and Chasseaud, 1969).

Cancer

Uncontrolled proliferation of cells, their invasion into their surrounding normal tissue leading to its destruction, and metastasis establishing new foci of growth are the three basic and well-known characteristics of malignent tumor.

Cancer as a disease, has existed all along with man. Hippocrates, twenty five centuries ago, called it Karkinois because the swollen blood vessels going and coming from the tumor mass, gave the appearance of the claws of crab. Susruta described cancer as a tumor which would ulcerate and would not cure and "sow its seeds in other parts of the body" (Jaggi, 1990).

All of the various cell types of the body can give rise to cancer cells. Cancer cells are usually closer in their properties to immature normal cells than to more mature cell types. They respond abnormally to the control mechanisms that regulate the division of normal cells, and they continue to divide due to genetic alteration in a relatively uncontrolled fashion untill they kill the host (Temin, 1970; Pierce *et al.* 1978 and Mderano and Pardee, 1980). Cancer can be thought of as a "wound that does not heal" (Beauchamp *et al.*, 1989).

Susceptibility to cancer is a polygenic phenomena. In addition, the influence of non-genetic factors such as hormones, nutritional status or chronic inflammation may modulate the development of neoplasia in a manner parallel to the phase of initiation and promotion seen in chemical carcinogenesis (Miller, 1980).

It is now generally recognized that most exogenous carcinogens require metabolic transformation into an active form and that the ability to activate these substances varies widely from individual to individual (Miller, 1980). Similar genetic control may be operating with regards to endogenous carcinogens as well (Lewan and Reilly, 1974). A precise parallel exists

between the metabolism of exogenous carcinogens and the metabolism of pharmacologic agents. A limited immune deficiency may also play a role in the etiology of primary hepatocellular carcinoma (Larouze *et al.*, 1977).

Furth (1975) hypothesized that hormones are not direct cacinogens but are indispensable components in carcinogenesis. The hormones enhance cell division and thus, favour the somatic mutation or unmasking mutations brought about earlier in response to carcinogens, in effect acting as promoters. The studies in mice showing the enhancing effect of hormones on breast cancer induction, supports this view (Bittner, 1957). Dyer *et al.*, (1975) have shown that hypersensitive patients carry higher risk of cancer development under certain circumstances.

Cancer can arise as the result of exposure to a variety of agents, studies have revealed that pure chemicals themselves are able to produce cancer (Millor, 1978). It is generally accepted that a high proportion of human cancer is attributed to environmental agents, mainly chemicals. The distribution of carcinogens in the environment is essentially ubiquitous. The human diet contains a variety of naturally occuring mutagens and carcinogens (Ames, 1983). The N-nitroso compound form a large group of agents, occur widely in the environment (Bartsch and Montesano, 1984). Many of them are carcinogens in experimental animals (Bogovski and Bogovski, 1982 and Magee *et al.*, 1982) and are causative agents in some human cancer (Bartsch and Mostesano, 1984). Major percentage of N-nitroso compounds are alkylating agents which react with nucleic acids and other cellular molecules

and exert many of their biological effect as a result of the transfer of an alkyl group (Millor, 1978; Magee *et al.*, 1982 and O'Connor *et al.*, 1979). Majority of chemical carcinogens are known to form a covalent adducts with DNA, thus indicating DNA as a critical target in chemically induced cancer (Millor, 1978; O'Connor, 1981). Colon and digestive tracts are exposed to variety of carcinogens derived from the rancidity of fat (Simic and Karel, 1980; Bisckoff, 1969; Petrakis *et al.*, 1981; Imai *et al.*, 1980; Ferrali *et al.*, 1980).

Hydrogen peroxide generated by the oxidation of dietary fatty acid by peroxisomes, is a known mutagen and carcinogen (Reddy *et al.*, 1982 and Plain, 1955). Some hydrogen peroxide may escape in the peroxisomes and contribute to the supply of oxygen radicals (Speit *et al.*, 1982 and Jones *et al.*, 1981), which in turn can damage DNA and can start the rancidity chain reaction, leading to the production of the mutagens and carcinogens (Pryor, 1976-1982).

One of the theories of etiology of cancer which is being widely accepted, holds that the major cause of damage to DNA is by oxygen radical and lipid peroxidation (Ames, 1983 and Totter, 1980). Certain promoters of carcinogenesis act by generation of oxygen radicals. Fats and H_2O_2 are among the most potent promoters (Welsch and Aylsworth, 1983). Other well known cancer promoter are lead, calcium, phorbolesters, asbestors and various quinones. Many carcinogens which require the action of promoters and by themselves are able to induce carcinogenesis (Complete carcinogens), also

produce oxygen radicals (Demopoules *et al.*, 1980). These include nitroso compounds, hydrazines, quinones and polycyclic hydrocarbons. Much of the toxic effect of ionizing radiation damage to DNA is also due to the formation of oxygen radical (Totter, 1980).

Recent advances in molecular biology have led to the concept that carcinomas arise from the accumulation of a series of genetic alterations involving activation of proto-oncogens and inactivation of tumor suppressor genes. p53 is a tumor suppressor gene located on chromosome p13 and mutation at this locus are the genetic abnormalities most frequently found in a variety of human malignancies, including gynaecologic cancer (Fearon *et al.*, 1987; Okamato *et al.*, 1991; Marks *et al.*, 1992; Milner *et al.*, 1993; Nigra *et al.*, 1989' Takahashi *et al.*, 1989).

Breast cancer is by far the most frequent cancer in women, and ranks third overall when both sexes are considered together. It is the most common cancer of women in all the "developed" areas (except for Japan, where it is second to stomach cancer) (Parkin *et al.*, 1993). Several studies about breast cancer indicated that this disease is the result of a combination of factors, such as ionizing radiation, diet, socioeconomic status psychosocial stress and endocrinologic, familal or genetic (Mathew *et al.*, 1990).

However, these pieces of information do not provide a complete picture of the pathogenesis of the disease, or of the mechanisms of interaction of the carcinogen with the target organ (Russo *et al.*, 1986 & 1987). Therefore,

we still lack effective strategies for breast cancer prevention and cure.

Beside breast cancer, liver cancer is considered one of the major cancers of developed and developing countries (Parkin, *et al.*, 1993). A number of reports have described the occurance of liver cell adenomas in women using oral contraceptives (James *et al.*, 1980). Circumstantial evidence derived from human and early experimental animal data, together with the reports of Tapper (1978) suggested that oral contraceptive steroids may be liver tumor promoters.

A number of studies have domonstrated many similarities between the pathogenesis and morphological changes of experimental and human liver cancer (Thomac, 1961 and Butler, 1971). Evidence that specific environmental chemicals from industrial, medical, and dietary sources are carcinogenic to the human has now become quite clear (Emmelot, 1977). Furthermore, there appears to be a role for at least one virus, the hepatic B virus, in the induction of human hepatic cancer (Tatematsu, *et al.*, 1977). There is a functional evidence for the multiple stages in the natural history of human hepatocellular carcinoma, some data point to a marked degree of similarity in hepato carcinognesis in the experimental animal and in the human beings (Henry, 1980).

Apart from uncontrolled proliferation of cells, their invasion into surrounding normal tissue, and metastasis establishing new foci of growth, the other adverse effects of the tumor on the structure and function of host cells that are not in direct contact with the tumor cells are reflected in altered

enzymes activities, metebolism, nutrition or hormonal imbalances and composition of blood. These changes are called paraneoplastic syndromes or tumor-host relations (Begg, 1958 and Hall, 1974). The well known examples of paraneoplastic syndromes are anorexia, catchexia, hypercalcemia of malignancy and anemia.

"It has been estimated that 20% of a group of patients at any time suffer from paraneoplastic syndrome at all stages of the disease and 75% of all patients will aquire one during the course of their disease". In some cases "disability or death will occur due to the syndrome than cancer per se" (Hall, 1974).

An interesting aspect of paraneoplastic syndrome is the mechanism by which they arise, i.e., the mechanism by which the tumor influences the host cells that are not in contact with it. Studies on paraneoplastic syndromes date back to earlier decades of this century. It has been shown that the liver catalase levels were greatly reduced in tumor-bearing animals and cancer patients (Begg, 1958; Hall, 1974; Bluementhal, 1910; Rosenthal, 1912 and Brahn, 1914).

The isolation stress is known to increase the activity of enzymes reponsible for metabolic activation of carcinogens, without influencing their excretion, and may therefore adversely affect carcinogens (Capel and Williams, 1979). The stressful situations are known to increase plasma cortisol levels also (Capel and Williams, 1979). Plasma cortisol level is found significantly raised in cancer breast and liver and the increase is more

pronounced in the patients with distant metastasis than in the tumor patients without distant metastasis (Schaur *et al.*, 1979; Khataibeh, *et al.*, 1996). Urinary excertion of 17-ketosteroids, cortisol sulfate and cortisone sulfate is found enhanced in cancer breast patients (Stancakova and Klimesova, 1979). Zeidman (1962) and Fidler and Lieber (1972) have reported increase in the incidence of metastasis following intravenously injected tumor cells in animals treated with corticosteroids.

Two to three fold increase of LDH is associated with fall of plasma cholinestrase level. This relationship is most marked in lymphomas. Further fall in plasma cholinestrase level is found to indicate that the disease is fairly advanced and has probably spread to the liver (Ghooi *et al.*, 1980). The elevation is highly marked in various cancers and more marked in primary and secondary liver cancer and certain other enzymes are elevated in the gastric juice of patients with gastric carcinoma (Smyrniotis *et al.*, 1962).

Decreased activities of monoamine oxidase, cytochrome oxidase, succinic dehydrogenase are found in both benign and malignant neoplasma and also in hyperplastic lesions (Wattenberg, 1974; McGinty *et al.*, 1973 and Banu *et al.*, 1988). Generally, the activities of some enzymes which are not essential for the process of rapid growth of the tissues are decreased in such situations.

Lower concentrations of tissues c-AMP have also been reprted in cancer breast (Patel *et al.*, 1981), alongwith enhanced catecholamine levels

(Udupa *et al.*, 1980; Patel, 1981, Singh. 1981; and Khataibeh, *et al.*, 1996)

Serum lactate dehydrogenase level has been found enhanced in all the cases of cancers (Ts'ao, *et al.*, 1996). Lactate dehydrogenase (LDH), serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) have been reproted as tumor marker enzymes (Vinitha, *et al.*, 1995). Cancer patients whose serum LDH was above the normal range or had not been normalized after 3 months of therapy were in the high risk group, and should be given more aggressive treatment (Masukagami, *et al.*, 1996). Tumor cells appear quite resistant to oxidative stress. Cell damage precipitated by protease, elastase and Triton probably involves hydrolysis of protein and phospholipids in the cell membrane, leading to an increased leakage of intracellular protein such as LDH (Ts'ao *et al.*, 1996).

Besides LDH, SGOT and SGPT, Glutathione-S-tranferase (GSTs) have also been reported as tumor marker enzymes (Tatemasu *et al.*, 1988; Vinita, 1995).

Glutathione-S-transferases (GSTs) as detoxificants and metabolizing enzymes have been linked with the susceptibility of tissues to environmental carcinogens (Stein, 1996). A hepatic GSTs are involved in hepatic detoxification and are considered to play important role(s) in chemical carcinogenesis (Arias *et al.*, 1976; Chasseaud, 1979; Jakoby, 1980 and Mannervik and Jenson, 1982). Vahrmeijer (1996) observed a lack of glutathione conjugation in human during cancer. However, changes in GST forms during chemical hepatocarcinogenesis have not been fully investigated.

It is established beyond doubt that free radicals in tissues and cell can damage DNA proteins, carbohydrates and lipids. These potentially deleterious reactions are at least partly controlled by antioxidants capable of scavenging radicals. It is widely belived that a proper balance between free radicals and antioxidants is essential for the health of an organism (Rautalahti, *et al.*, 1994). Reactive oxygen species and other free radicals are known to be the mediators of phenotypic and genotypic changes that lead from mutation to neoplasia. In erythrocyes reactive, oxygen species and other free radicals can result to hemolysis which is one of the pathogenetic mechanisms of anemia in cancer patients (Zima *et al.*, 1996)

Free radicals and reactive oxygen metabolites due to increased production or reduced inactivations, following a decrease in the antioxidant burden in the mucosa, might cause damage to DNA, thereby resulting in genetic alterations. This might represent the cause of the transformation process (Pappalardo *et al.*, 1996). Apart from oxidants (free radicals), cigarette smoke contains such a multitude of (pre) carcinogens that it is astonishing that not every heavy smoker becomes a victim of malignancy. This points to the interindividual variability in susceptibity to carcinogens; several lines of evidence suggest that metabolic and personality factors are involved in such variability (Russo *et al.*, 1987). Metabolism of carcinogenes as well as the subsequent (multi) steps of carcinogenesis are affected by host factors and governed by the balance between opposing forces, such as metabolic activation and detoxification, formation and scavenging of radicals and DNA

activation and detoxification, formation and scavenging of radicals and DNA damage and repair, which seem to imply that carcinogenic compounds can initiate tumor growth only in amount saturating detoxification mechanisms. In this context it is well known that reduced glutathione (GSH) plays a critical role in the detoxification process, which is reported to be a safe agent without major side effects and has been emerged as a most promising cancer chemopreventive agents (Van Zandwijk, 1995). Some workers have implicated the loss of antioxidant glutathione in the pathogenesis of parkinson's disease (Drukarch *et al.*, 1996).

Antioxidant, vitamins which include beta carotene, vitamin E, vitamin C and related micronutrients are hypothesised to decrease cancer risk by preventing tissue damage by trapping organic free radical and/or deactivating excited oxygen molecules, a by-product of many metabolic functions (Hennekens 1994) where the lowest fruits and vegetable intake has been consistently associated with increased risk of cancer (Van Zandwijk, 1995; Romeny 1995; Bowen and Mobarhan, 1995; Schwartz 1996). But much less evidences show that such low intake can encourage the development of cancer which are under hormonal control (Schorah 1995).

Erythrocytes have a life span of about 120 days, Smith (1995) reported that exercise, cycling, running and swimming have been shown to cause RBC membrane damage. The neutral amino acids are found to reduce the hypotonic hemolysis at pH 5.0 but enhance it at pH 8.0 (Morimoto *et al.*, 1995). Thus, these amino acids controlled the osmotic fragility of cell membrane showing a protective effect

The osmotic fragility test is used to determine the extent of red blood cell hemolysis produced by osmotic stress. Erythrocyte hemolysis is dependent upon cell volume, surface area, and functional integrity of cell membranes. The dependence of RBC hemolysis on concentration of sodium chloride has been determined spectrophotometrically by measuring the absorbance of released hemoglobin (Orcutt *et al.*, 1995). Incubation of RBC with lactic acid for one hour at 37 °C increased the osmotic fragility of erythrocytes. Even in the absence of lactic acid, RBC subjected to heat shock at 42 °C showed increased osmotic fragility as compared to 37 °C (Kogawa *et al.*, 1995).

Reactive oxygen species and other free radicals can cause erythrocytes hemolysis, which is one of the pathogenic mechanisms of anemia in cancer patients. In multiple myeloma patients, the activities of superoxide dismutase and glutathione peroxidase were significantly lowered. These results proposed a possible role of free radicals with reduced antioxidant activities of SOD and glutathione peroxidase (GPs) in multiple myeloma (Zima *et al.*, 1996).

Stress and Cancer

Interest in the involvement of personality factors or stress in the evolution of human cancer dates back to 175 A.D. when Galen stated that 'Melancholy' women were more prone to cancer than their 'Sanguine' counterparts. Other workers also reported that grief and mental depression is associated with breast cancer (Cutter. 1954). Later Snow in 1893 found

that the occurance of malignant disease of the breast and uterus is preceded by a previous history of emotions of a depressive character. Further, Leshan and Worthington (1956) and Schmale and Iker (1971) have demonstrated a relationship between the malignant disease and preceding stress, several eminent, clinicians have reported that temperament, depression and life stresses appear to be related to the life development and course of cancer (Kowal, 1955; Leshan and worthington, 1956; Guy, 1967 and Snow, 1967). Moore (1969) suggested that stress and strain of modren life may also be one of the causes for oncogenesis. Moore (1969) proposed that sociological stress in women may be one of the factors in the etiology of breast cancer. Several other convincing evidences are there to incorporate the involvement of stress in the development of tumors (Seifter, 1976; Udupa *et al.*, 1980 and Banu *et al.*, 1988). Many workers have correlated the psychological factors with human cancer (Cobb, 1952; Cutter, 1954; Corson, 1966). Cholst, (1996) proposed that chronic stress in human can cause psychological and physiological reactions.

Riley (1975) observed that the incidence of mammary tumors in experimental mice could be increased to 90% by exposing them to a variety of stressors whereas the incidence in control mice was only 7%. Thus, he concluded that moderate, chronic or intermittent stress may predispose such mice to increased risk of mammary cancer and adequate protection from physiological stress may reduce mammary tumor occurance in mice. Chronic sterss can be lessened in the treatment of cancer (Cholst, 1996).

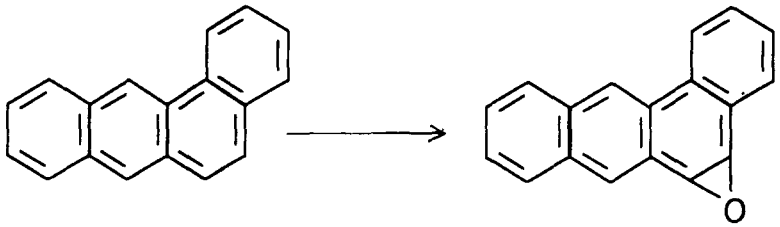
Malignant neoplasma undoubtedly elcit the typical manifestations of the G.A.S. both in animals and in man (McEuen and Selye, 1935; Moore *et al.*, 1969 and Ertl, 1973). Many types of cancer develop at sites of chronic local stress (Selye, 1979). According to Cole (1973), stress enhances metastasis in experimental tumor bearing animals. In susceptible persons, a severe emotional distress can trigger carcinogenesis, whereas various educational measures can inhibit the growth of cancer (Reichel, 1977). Various other workers have suggested that suppressed anger and difficult early relationship with parents could be one of the predisposing factors in the oncogenesis (LeShan, 1966; Thomas and Duszynski, 1974).

To establish a relationship between stress and cancer an experimental system is needed that mimics the human disease and therefore rats were considered as one of the most widely studied and useful models of mammary carcinogenesis (Dao 1962; Huggins 1959 and Young and Hallowes 1973). The commonly used strains, Spraque-Dawley and wistar-Furth are most susceptible to DMBA carcinogenesis. (Isaacs, 1986). Thus, Spraque-Dawley strain of rats were employed in the present study of cancer by infusion with 7,12-Dimethylbenz(a)anthracene (DMBA) (Rogers *et al.*, 1990).

DMBA is one of the most potent cancinogenic polycyclic hydrocarbons (Brookes and Lawley, 1964 and Slaga *et al.*,1974). Roger and Lee (1986) reported that the two most widey used experimental systems for the study of mammary tumorigenesis are the models in which tumors are induced in the Spraque-Dawely (S-D) rat by 7,12-Dimethylbenz(a)anthracene (DMBA), or in

the S-D or Fischer 344 rats by N-methylnitroso urea (NMU). DMBA, given by gavage in a single dose of 3.5-30 mg induces tumors with latencies that generally range between 8-12 weeks with and final tumor incidences close to 100% if sufficient time elapses before recropsy.

7,12-Dimethylbenz(a)anthracene, present in cigarette smoke and charcoal broiled foods is carcinogenic. Some carcinogens act directly, while other, such as benz(a) anthracene must undergo prior hydroxylation by arylhydroxylases, present mainly in the liver, before their carcinogenic potential can be expressed.



Benz(a)anthracene 5,6-Epoxide (Carcinogenic)

The susceptibility of the mammary gland to DMBA-induced carcinogenesis is strongly age-dependent and is maximal when the carcinogens are adminstered to animals between the ages of approximately 45-60 days, that is the age of sexual maturity (Grubbs. *et al.*, 1986 and Rose, 1980). Active organogenesis and high rate of proliferation of the glandular epithelium are characteristics of that period (Rose *et al.*, 1980). In virgin rats treated with DMBA, tumors that develop are largely carcinomas although the proportion can be altered by carcinogen dose and dietary fat (Chan *et al.*, 1983).

The administration of DMBA to rats of different ages induces tumors with an incidence which is directly porportional to the density of highly proliferating terminal end buds (TEB) (Russo *et al.*, 1978). A 100% incidence of carcinomas is obtained when DMBA is administered to rats aged 30-55 days, but with the highest number of tumors/animal is observed when the carcinogen is given to animals when they are 40 to 46 days of age (Russo *et al.*, 1990). Pregnancy occuring early after carcinogen exposure increases tumorigenesis (Grubbs, 1983). Caffeine increased mammary gland development in mice, apparently by increasing the response to trophic hormones (Welsch *et al.*,1988).

Epidemiologic studies strongly indicate that alcohol intake is a risk factor for breast cancer (Rogers *et al.*,1988). The increased risk is 1.3 to 3 folds depending upon the population studied and the amount of alcohol consumed (Barsky *et al.*,1984 and Geschicker, 1945). Vitamin A and related retinoids reduced mammary tumrigenesis (Aylsoworth *et al.*, 1986 and McCormick, 1981).

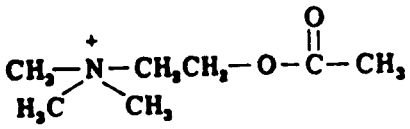
Acetylcholine strase(AChE) : (EC.3.1.1.7)

It is the enzyme which catalyses the hydrolysis of acetylcholine into choline and acetate and thereby inactivates the esters. The name acetylcholinesterase was proposed by Augustinsson and Nachmansohn (1949). One molecule of enzyme may split one molecule of ACh in about 3-4 microseconds. Extensive studies on the concentration and distribution of AChE in

conductive tissues have shown that significant amount of ACh may be split per gm of tissue within milliseconds, ie within a period of time which the impulse passes (Nachman Sohn, 1939). The concentration of enzyme is high in all nerve tissues. Nerve fibres are capable of hydrolyzing amount of ACh ranging usually from 5 to 50 mg per gm fresh tissue per hour. Alles and Hawes (1940) showed that erythrocyte esterase differ markedly from serum esterases. Richter and Croft (1942) confirmed that red cell esterase is highly specific for ACh. Zeller and Bisseger (1963) found that brain esterase is fundamentally similar to red cell esterase. The rate of hydrolysis of ACh was found to be optimal at about 6 to 8 x 10^{-3}M substrate concentration. Higher concentrations increasingly inhibit the rate of hydrolysis. The function of acetylcholine in RBC is unknown but Brauer and Root (1945) found the enzyme is localized in the surface of the red cells. The membrane environment of red cell is also important for the enzyme reactivity (Flis, 1979). Characterization and investigation of AChE with respect to its kinetics has been reported in W. aegyptia venom (Al Jafari *et al.*, 1995). AChE has also been isolated from rat liver (Mansee *et al.*, 1995). Several workers have reported the inhibition of anesthetics like halothane, methoxy flurane, di-ethyl ether, chloroform on erythrocyte bound AChE activity (Yoshimura *et al.*, 1995). N-Benzylpiperidine derivatives have been found to inhibit the metabolic breakdown of ACh via AChE, hence alleviating memory defects in patients with Alzheimer's disease by potentiating cholinergic transmission (Tong *et al.*, 1996).

Fig. 2 : Metabolism of acetylcholin.

Fig. 3 : Metabolism of histamine.



Acetylcholine

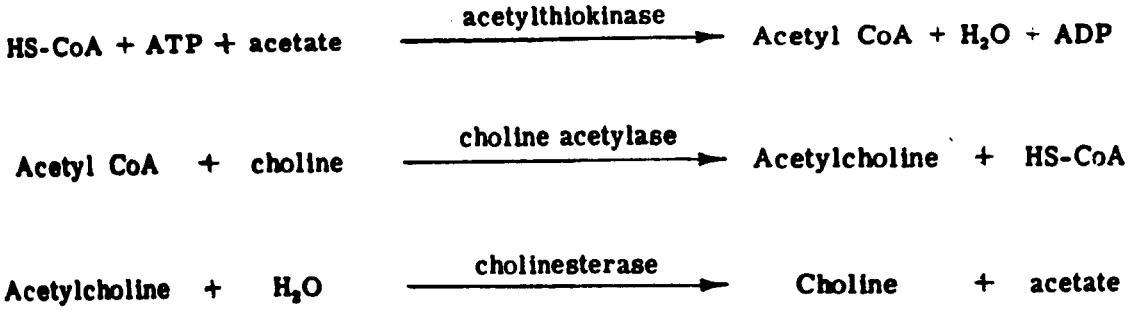


Fig. 2

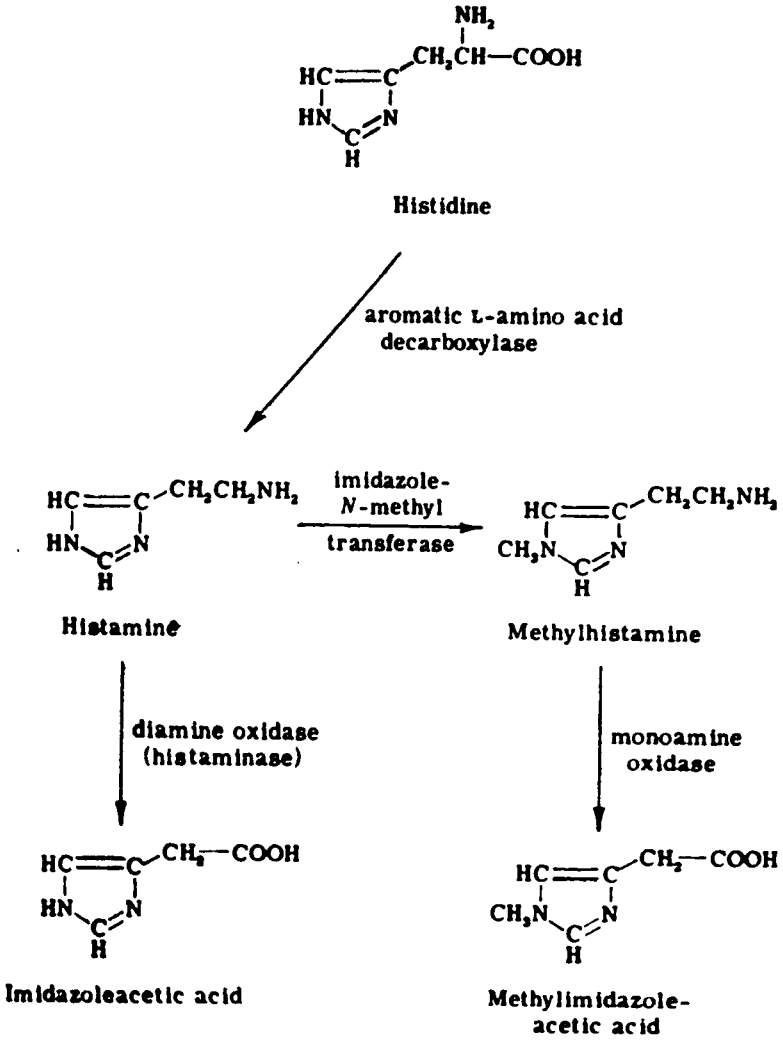


Fig. 3

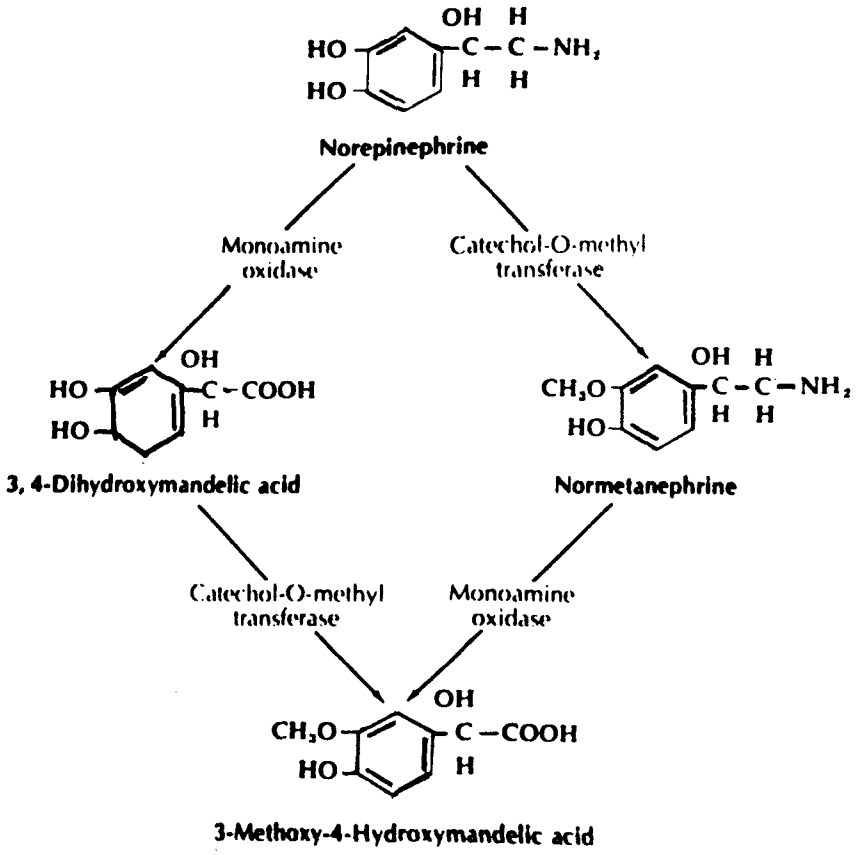


Fig. 4

Catabolism of catecholamine.

Monoamine Oxidase(MAO) : (EC.1.4.3.4)

Monoamine oxidase is a flavin-containing enzyme located on the outer membrane of the mitochondria (Cost and Sandler, 1972). It was first reported in liver by Hare (1928). Oxidative deamination of primary monoamines by the mitochondrial enzyme monoamine oxidase produces NH_3, aldehyde and H_2O_2, agents with established or potential toxicity (Cooper *et al.*, 1978; Benedetti and Dosteret, 1989).

MAO is one of the major mammalian neuronal enzymes. It is active in both neuron and glial cells in the brain. MAO plays a strategic role in inactivating catecholamines that are free within the nerve terminals and not protected by the storage vesicles (Coyle and Synder, 1981). When monoamines leak from the synaptic vesicles, MAO acts within the nerve fibre itself. The concept of two distinct form of MAO has gained wide acceptance (Johnston, 1968; Houslay *et al.*, 1976 and Leung *et al.*, 1981). Type A deaminates neurotransmitter amines such as 5-hydroxytryptamine (5-HT) and noradrenaline (NA) and is inhibited specifically by glergyline, whereas type B oxidizes benzylamine and β-phenylethylamine and is preferentially inhibited by deprenyl phenyl isopropyl methyl propinyl amine (Tipton and Della Corte, 1979). Both form deaminate substrate such as dopamine, tyramine and tryptamine (Houslay *et al.*, 1976).

However, both forms of the enzymes are not found in all the tissues (Fuller and Roush, 1972). The deamination mechanism for both types are the same.

$$RCH_2NH_2 + O_2 + H_2O \xrightarrow{MAO} RCHO + NH_3 + H_2O_2$$

One mole of O_2 is required for the oxidation of one mole of substrate. MAO acts only on those which have an amino group attached to the terminal carbon atom (Blaschko, 1952). When the amino group is directly attached to the benzene ring, deamination does not occur, but terminal carbon atom of the amines as in amphetamine and epinephrine, they are not metabolized by MAO, MAO is knwon as the principal enyme taking part in the deamination reactions (Yasunobu *et al.*, 1968). Generally all the vertebrates contain MAO. The usual sources of MAO are the mitochondria of liver, brain (Weiner, 1960), adrenal gland, kidney and peripheral and central nervous system (Blaschk *et al.*, 1955). It is also found in heart, salivary gland, spleen, and noradrenergic granules (Tripton *et al.*, 1976).

Inhibitors of MAO play a significant role in the metabolism of catecholamine, serotonin and other amines (Zellet *et al.*, 1952). The first monoamine oxidase inhibitor i.e. hydrazine was found in 1952. The use of such inhibitors increases the concentration of neurohumors and decreases the excretory metabolities like VMA and HVA. However, the concentration of normetanephrine, metanephrine and octopamine are increased following administration of MAO inhibitors (Sjoerdona, 1970). The evidence that the two forms of MAO result from differences in membrane lipid binding suggests that the activity of the enzyme *in vivo* might be affected by drugs and diseases that affect lipid metabolism.

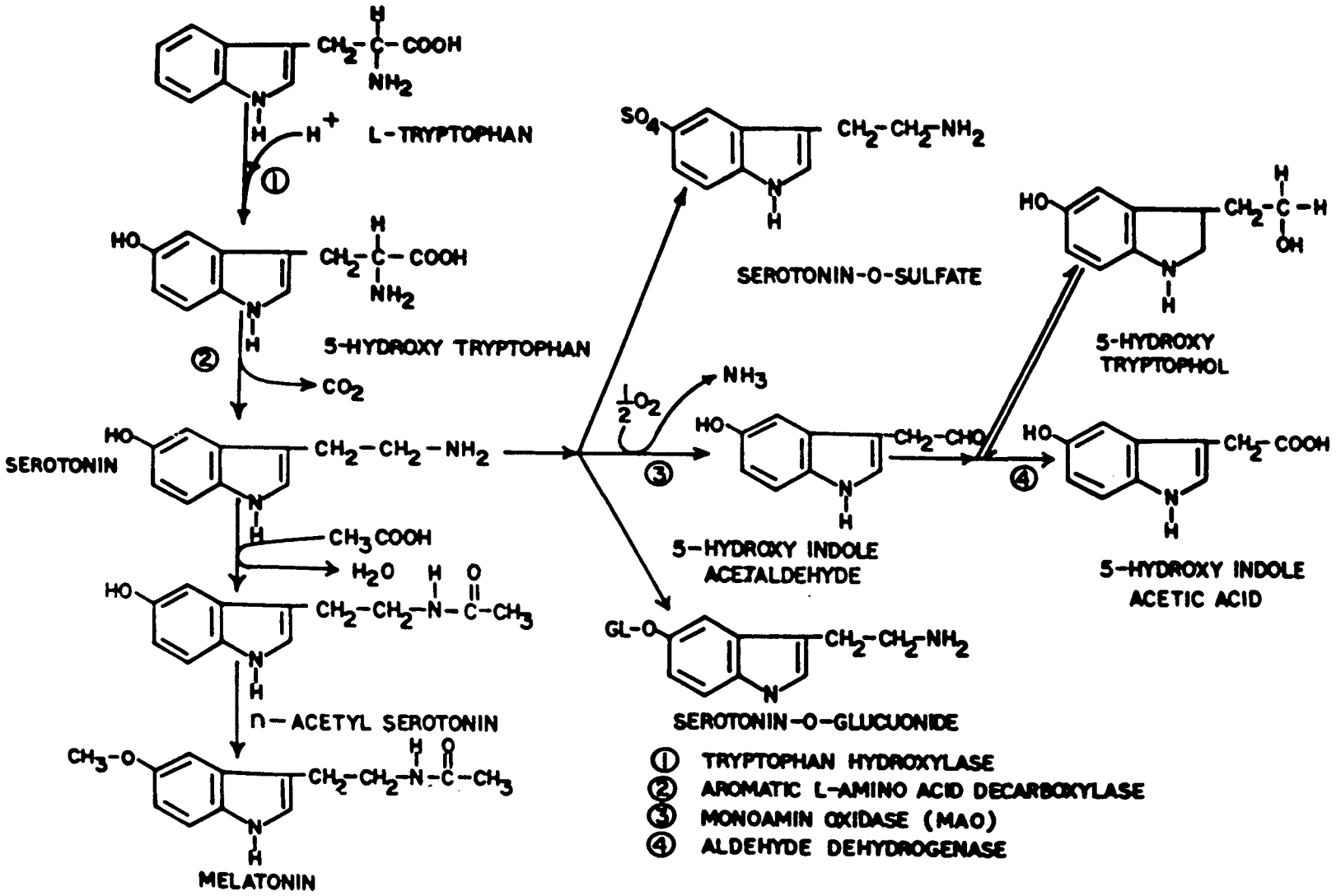


Fig. 5

Fig. 5 : Metabolism of 5-hydroxy tryptamine.

Cortisol

Cortisol is a corticosteriod hormone, which is secreted from the adrenal cortex. The hormones of the pituitary-adrenocortical axis are involved in the regulation of functions of the central nervous system (Holsboerm, 1989). They not only coordinate the neuroendocrine processes to stress itself, but also affect psycho-physiological processes. In 1936 Selye observed that diverse noxious agents cause an enlargement of the adernal cortex as a consequence of the "stress syndrome". Yates and Maran (1974) reproted that a variety of stressful events cause a release of ACTH from the anterior pituitary. The secreted ACTH stimulates the synthesis of corticosteroids in the adrenal cortex. The elevated corticosteriod levels in plasma then inhibit the further release of ACTH from the pituitary. In a series of elegant experiments, Harris (1948) demonstrated that the release of ACTH from the pituitary is regulated by a corticotropin-releasing factor (CRF) from the hypothalamus. The CRF is synthesized in the hypothalamus. The CRF synthesized in the hypothalamus reaches the pituitary by a private portal blood supply. It then stimulates the secretion of ACTH from the pituitary. After a long period of intensive investigations, CRF was isolated and purified, and its structure was characterized as a 41 amino acid peptide by Vale and co-workers (1981). CRF was thought to be the major, if not the sole means, of releasing ACTH from the pituitary. ACTH can also be released and regulated by catecholamines and other hormones (axelrod and Reisine, 1984).

There have been a number of investigations using cortisol to assess the reaction of the pituitary-adrenocortical axis under various conditions. Lundberg and Frankenhauesr (1980) found increased cortisol levels in situations which were accompanied by boredom, impatience and tiredness (vigilance task). In situations charactrized by a high controllability and predictability (self-placed RT-task), Lehmann *et al.*, (1992) reported an adrencortical suppression. Furthermore, there is increasing evidence that cortisol modulates brain function in humans. This principal endogeneous glucocorticoid in humans increases slow-wave sleep and decreases rapid-eye-movement sleep (Born *et al.*, 1991). There is some evidence that heart rate changes are accompained by cortisol changes dependent on personality. Furthermore, an increasing heart rate is related to increasing difficulty of a task (Eason and Dudley, 1971; Carrol *et al.*, 1986).

Glutathione

Sulfhydryl (-SH) group is also known a thiol group. It plays a key role in active enzymatic sites of many important enzymes (Hoch and Vallee, 1959). In principle, any enzyme bearing an accessible thiol, essential for activity is capable of forming protein mixed disulfides or intramolecular disulfides by reacting with small disulfides. Formation of mixed disulfides or intramolecular disulfides can increase or decrease catalytic activity and examples of both are known. Furthermore, the extent of enzymes-S-thiolation would depend on the thiol-disulfide redox potential as well as the nature of the small disulfide and the micro-environment

around the accessible protein thiol. These parameters are at least potentially capable of conforming the specificity required for a biological control mechanism through signal transmitted by changes in the thiol-disulfied redox potential as function of different matabolic states.

Glutathione (GSH) protects hemoglobin and other critical erythrocyte proteins from preoxidative injury. Sulfhydryl groups derived from the side chain of cysteine residues, occur in a number of enzymes. Sulfhydryl (-SH) group and disulfide (-SS) bond of cysteine are highly reactive and are apparently involved in the maintenance of the conformation and biological activity of certain proteins. As the receptors are protein in nature, the reagents, which modify -SH groups may influence the interaction of neurotransmitters with their recognition sites (Sobrino and Del Castillo, 1972).

Sulfhydryl groups play an important role in GST induced detoxification against eletrophilic xenobiotics and toxicants by conjugating with such compounds and thus neutralizing their electrophilic sites (Habig *et al.*, 1974).

Glutathione has been considered to function as biological antioxidant. It plays a pivotal role in the destruction of free fadical as well as inorganic and organic peroxides (Sohal *et al.*, 1984). GSH is a naturally occuring and widely distributed tripeptide. It consists of glycine, cysteine and glutamic acid moieties (Allen and Balin, 1989). γ-glutamyl cysteinyl glycine molecule is the major nonprotein thiol compound present in cells in concentration which range between 0.1 and 10 mM (Kosower, 1976a). It is synthesized intracellularly by the consecutive action of glytamyl cycteine synthase and GSH synthase. Its concentration is dependent

on metabolic rate and the level of oxidative stress (Allen and Sohal, 1986). It has been implicated in a wide variety of biological functions, such as the maintenance of all membranes, destruction of metabolic peroxides and free redicals, detoxification of foreign compounds, removal of H_2O_2 maintenance of thiol group of enzymes and proteins, control of redox status, disulfide exchange reaction, transport of amino acids and peptides across membranes (Hazelton and Lang, 1980; Meister and Aderson, 1983 and Ziegler, 1985) besides the presence of low pH, lactate is the main factor causing depletion of intracellular glutathione (Breborwicze *et al.*, 1996)

Glutathione-S-Transferase (GST) : (EC. 2.5.1.18)

Glutathione-S-tranferase is a non-selenium dependent glutathione peroxidase (Sies *et al.*, 1979). GST was first identified in 1961 (Booth *et al.*, 1961 and Coombs and Stakelum, 1961). The enzyme was subsequently named glutathione-S-aryl transferase. Later on, several other GSTs were demonstrated depending upon the substrate specificity.

Among many enzymes discovered to be important for toxicity of chemicals in humans, glutathione-S-transferases are of most interest in occupational toxicology (Goergens, 1996). The enzymes are almost ubiquitous in nature, and their activities have been identified in man, non-human primates, rats, mouse, hamster, guinea pig, chicken, cow, sheep, troup and shark (Mannervik, 1985). The concentration of GST is in general, high in mammals (upto10% of cytosolic proteins in some organs), in other species

(shark) the level of activity is quite low (sugiyama *et al.*, 1981). In addition, it is generally present in most mammalian organs.

The GSTs are a family of multifunctional protein that function both as important enzymes of detoxification and intracellular binding proteins (Boyer, 1989). At least six forms of rat liver GST have been characterized by physiochemial properties (Arias *et al.*, 1976; Chasseaud, 1979; Jakoby, 1980; Mannervik and Jensson, 1982 and Pabst *et al.*, 1974). As enzymes, they catalyze the reaction between nucleophil reduced GSH and large number of electophilic compounds such as polycyclic aromatic hydrocarbons, aromatic amines, azo dyes, alkylating agents, carcinogens and neurotoxins (Boyland and Chasseand, 1969; Habig *et al.*, 1974; Jokoby, 1978 and Chasseand, 1979). They also bind a number of amphipathic compounds that they do not metabolize (non-substrate ligands) and have been suggested to act as intracellular transport proteins for compounds that have limited solubility in water (Levi *et al.*, 1969).

Superoxide Dismutase (SOD) : (EC 1.15.1.1)

All aerobic organisms utilized O_2 and must have some mechanism by which they can minimize O_2 toxicity. One mechanism is the production of sueroxide radical and its dismutation reaction, catalyzed by the enzyme superoxide dismutase (Harman, 1956 and 1971). The superoxide anion is a free radical formed by one electon transfer to oxygen.

$$O\cdot + e^- \rightarrow O_2\cdot^-$$

Superoxide dismutase (SOD) catalyzes the dismutation between two moles of

superoxide anion to yield one mole of oxidized product (oxygen) and one mole of reduced product (hydrogenperoxide) (Klug *et al.*, 1972).

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2$$

This is analogous to the dismutation of hydrogen peroxide to oxygen and water catalyzed by catalase ordinarily, electrostatic repulsion between two molecules of superoxide anion limits their approach to one another; SOD overcomes the barrier and greatly increases the dismutation rate (Fridovich, 1976 and 1978).

Several forms of SOD have been identified since the enzyme was first discovered in 1969 by McCord and Fridovich. They identified the enzymatic activity associated with erythrocuperein, a copper-zinc protein of erythrocytes. The copper is associated with enzymatic activity, whereas the zinc is structural. Similarly, SOD activity is associated with a family of Cu-Zn proteins, cerebrocuprein in brain (Fried, 1979) and hepatocuprein of liver. In mammalian tissues, a second form exists in which manganese is the prosthetic group (Fridovich, 1976). In rats and mice the Mn-SOD is localized to mitochondria, whereas Cu-Zn SOD is cytoplasmic. However, this distribution does not hold in other species.

Fried and Mandel (1975) indicated that very high levels of activity are present in liver, while the adrenals, kidney and red blood cells have intermediate activity and lower activities were found in most other tissues including brain. Regional distribution studies in the rat by Thomas and his co-workers (1976) showed a relatively homogenous distribution in brain, about a two-fold range from the highest area (medulla oblongata) to the lowest area (cortex). Subcellular distribution studies in the rat (Thomas *et al.*, 1976) showed the highest level in the cytoplasm while myelin has very low levels.

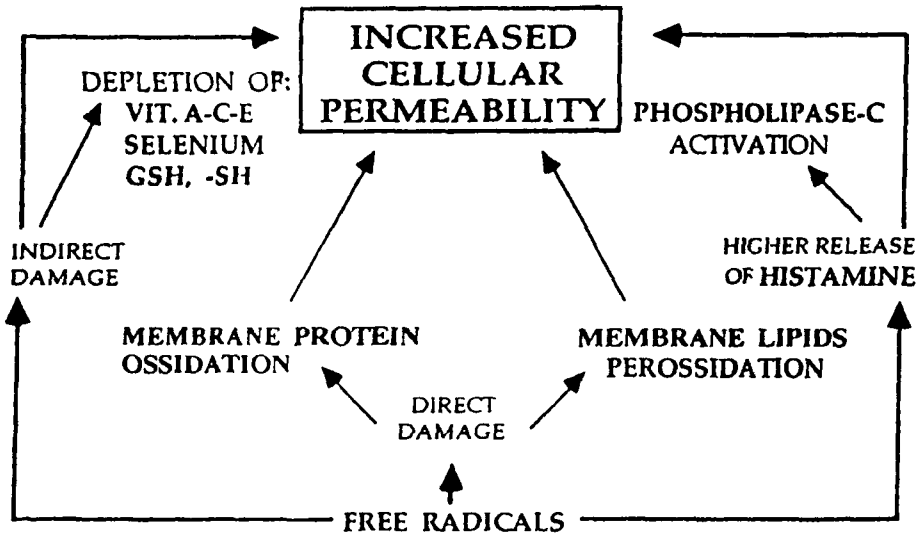
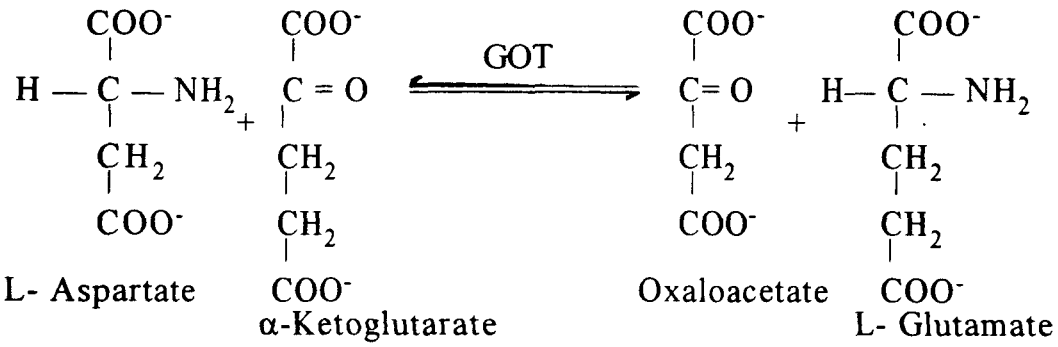


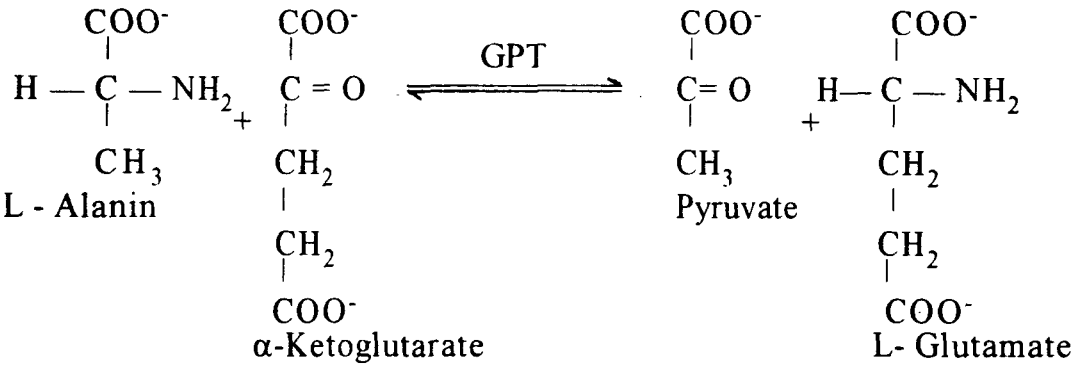
Fig. 6

Action mechanism of free radicals on membrane permeability.

Transaminases (*Aminotransferases*)

The transaminases constitute a group of enzyme that catalyze the interconversion of amino acid and α-ketoacid by transfer of amino group. The α-ketoacidglutarate/L-glutamate couple serves as one amino group acceptor and donor pair in all amino trasfer reaction; the specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group. Thus, aspartate amino transferase (GOT): (EC. 2.6.1.1) and alanine amino transferase (GPT): (EC. 2.6.1.2) catalyze the reactions as follows:





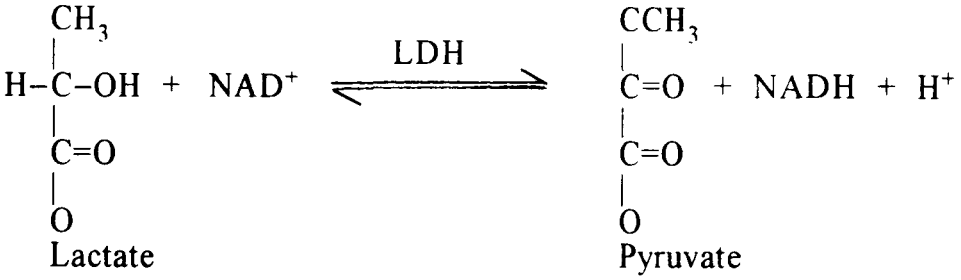
The reactions are reversible, but the equilibria of the GOT and GPT reactions favor formation of the Aspartate and alanine respectively.

Transaminases are widely distributed in animal tissues.

Asparate transaminase (GOT) have been isolated from the thermophilic microorganism Bacillus isothermophillus (Bartsch Klaus *et al.*, 1996). In viral hepatitis and other forms of liver diseases associated with hepatic necrosis, serum GOT and GPT are found elevated even before the clinical signs and symptoms of disease appear (e.g. jaundice). Five to ten fold elevations of the two enzymes occurs in patients with primary or metastatic carcironmas of the liver, with GOT usually being higher than GPT. (Vinitha *et al.*, 1995). Rapid increase in the activities of the two enzymes in serum have been reported during restraint stress (Sun *et al.*, 1995) and a slight or moderate elevations of both SGOT and SGPT activities may be observed after intake of alcohol and after administration of a variety of drugs (e.g. ampicillin) (Norbert, 1996).

Lactate dehydrogenase (EC.1.1.1.27)

Lactate dehydrogenase is a hydrogen transfer enzyme that catalyzes the oxidation of L - Lactate to pyruvate with the mediation of NAD+ as hydrogen acceptor. The reaction is reversible and the reaction equilibrium strongly favours the reverse reaction, i.e. reduction of pyruvate to lactate.



Two isozymes (LDH -5 & LDH - 4) of lactate dehydrogenase have been purified 21 fold from the liver of the reptile Varanus bengalensis (Masood *et al.*, 1997). Lactate dehydrogenases are inhibited by reagents with reactivity against thiol groups such as mercuric ions and p-chloro mercuribenzoate. Inhibition can be reversed by the addition of cysteine or glutathione. LDH activity is present in almost all cells of the body. Enzyme levels in various tissues are very high compared to serum. Serum levels of LDH were found elevated at 2 hours and increased continously upto 8 hours of restraint stress (Sun *et al.*, 1995). LDH have been proved as tumor marker for their changes in concentration in serum, liver and kidney (Vinitha *et al.*, 1995). Exposure of rats to hypoxia produced a proportional loss of body and heart weight with an equal decrease in both LDH subunits H (heart) and M (Muscles) (Kaaja and Ari, 1996). Pleural fluid LDH isoenzyme pattern may be helpful for the differential diagnosis of the most common causes of pleural effusions; congestive heart failure, infections and malignancy (Lassos, Izidores *et al.*, 1997). Elevation of LDH activity was observed in liver disease, but these elevations are not as great as the increases seen in transaminase activity.

Indigenous drugs :

Sage (Salvia officinalis *Labiatae*)

Medicinally, Sage is used as a mild tonic, a stringent and carminative. An infusion of the leaves is used as a gargle in the

Fig. 9 : Sage (Salvia *Officinalis*).

treatment of sore-throats; hot infusion is said to be diaphoretic. Extracts of sage leaves are also reported to be antipyretic (Sharma, 1965). In Chinese medicine it is a yin tonic with a well deserved reputation as a nerve tonic helping both to calm and stimulate the nervous system (Andrew, 1996). Sage is a valuable remedy for female disorders since ancient times. Though its hormonal action is not completely understood, there is no doubt it reduces sweating, which is coupled with its tonic and estrogenic effects, making it an excellent remedy for the meno-pause, not only reducing hot flushes, but helping the body to adapt to the hormonal changes involved (Andrew, 1996). The estrogenic substances have been extracted from the dried leafy tops (Hanson and Hocking, 1950 and Sastri, 1956).

Sage has a slight warm, noticeable bitter and astringent taste. It is rich in flavonoids and phenolic acids (Andrew, 1996). The leaves of sage contain an essential oil. Small amounts of triterpenoids and steroids are also reported to be present in its oil (Sharma, 1965).

Sage and sage oil exhibit antioxidant properties, five antioxidant fractions with antioxidant indices between 8.8 and 10.0 have been isolated from leaves of *S. officinalis*, one of which appears to be a polyhydric phenol (The Sastre, 1956). Edible plants containing variety of substances such as phenols and flavonoids (Nagao *et al.*, 1977), have a wide spectrum of pharmacological properties (Bertz *et al.*, 1977), have been reported to inhibit

carcinogenesis and mutagenesis in experimental animals (Ames, 1983 and VanHoff *et al.*, 1984), and implicated as novel antiviral (Dawson, 1934). Flavonoids have been reported to have antioxidant properties and act as scavengers of free radicals (Puppo, 1992). The inhibitory action of such compounds may be due to the induction of cytochrome P-450 and other metabolic enzymes (Boyd *et al.*, 1982)

Garlic (Allium sativum *Liliaceae*) :

Known for its pungent odour and taste, garlic is an ideal herbal medicine, being completely safe for home use and a powerful treatment for a host of health problems.

Selenium, Vits A, B, C and E, organosulfur compounds i.e. diallyl disulfide (DADS) S-allyl cysteine (SAC) and related compounds, like diallyl trisulfide (DATS) are some of the important components of garlic (Lea, 1996; Singh, *et al.*, 1996; Sundaram, *et al.*, 1996). Various dietary constituents may influence the incidence and severity of human cancer (Milner, 1989). However, the impact of these dietary contents is dependent on the composition of the entire diet. Although interactions between essential and nonessential ingredietns of the diet are recognized, their significance in the cancer process remains largely unexplored. There is also evidence that several antioxidant nutrients in human diet such as selenium, vits A, B, C and E and flavonoids in dietary plants have a protective effect against coronary heart disease and cancer

(Catherine, 1995), and chemically induced cancer (Thoumpson *et al.*, 1981).

Garlic (Allium Sativum), proclaimed for its medicinal properties for centuries appears to possess anticarcinogenic properties. Gastric and colon cancer risks are reported to be lowered in individuals consuming increasing quantities of garlic (Mei *et al.*, 1982 and Steinmetz *et al.*, 1994). Unfortunately, the impact of dietary garlic on other types of human cancer remains largely unexplored, Nevertheless, laboratory investigations with animals suggest that garlic may inhibit chemically induced cancers including breast, skin, forestomach, lung and colon (Liu *et al.*, 1992; Perchellet *et al.*, 1990; Sparnins *et al.*, 1988 and Sumiyshi and Wargovich, 1990).

Studies by Liu *et al.*, (1992) suggest that garlic inhibits both the initiation and promotion phases of 7,12-dimethylbenz(a) anthracene (DMBA) carcinogenesis. The organosulfur compounds including allyltri-sulfide, diallyl trisulfide, allylmethyldisulfide, diallyl disulfide (Sparnin *et al.*, 1988; Singh *et al.*, 1996) and selenium enriched garlic (Thompson, 1996) inhibited chemically induced tumors. Ip (1996) reported that selenium enriched garlic inhibits the early stage but not the late stage of mammary carcinogenesis.

Selenium is also recognized as an anticarcinogenic agent present in many plants. The protective functions are not only due to its action through glutathione peroxidase but it appears to operate by several

mechanisms depending on dosage and chemical form of selenium and the nature of the carcinogenic stress. Selenium is proposed to prevent the maligant transformation of cells by acting as a "redox switch" in the activation-inactivation of cellular growth factors and other functional proteins through the catalysis of oxidation-reduction reactions of critical SH group of S-S bonds. Selenium may also alter carcinogen metabolism and protect DNA against carcinogen-induced damage (Schrauzer, 1992).

Recent studies have shown that dietary garlic supplements provided to rats consuming a semipurified diet markedly suppressed the occurance of DMBA induced adducts bound to mammary cell DNA and inhibited the incidence of DMBA induced mammary tumors (Amagase and Milner, 1993 & Liu *et al.*, 1992). Amages *et al.*, (1993) have noticed that addition of garlic powder to rat's diet reduced the binding of DMBA metabolites to mammary cell DNA and reduced the incidence of DMBA-induced mammary tumors.

Amagase and Milner (1993) found that the different allyl sulfur compounds, S-allyl cysteine, accounted for much of the ability to inhibit DMBA induced DNA adducts. Because the quantity of DMBA metabolites bound to mammary cell DNA 24 hours after carcinogen treatment correlates with final tumor incidence, changes in adducts do serve as an early indicator of alterations in initiation phase of DMBA carcinogenesis (Liu *et al.*, 1991 and 1992 and Amagase *et al.*, 1996).

Except the effect of garlic on DNA, the detailed mechanism by which it inhibits the DMBA carcinogenesis remains unknown. However, changes in both phase I and II enzymes involved in carcinogen bioactivation and detoxification are recognized to occur in animals treated with various garlic preperations (Dalvi, 1992). In rats, tissue glutathione content and activities of glutathione-S-transferase have been shown to increase following consumption of garlic or related sulfer compounds (Sparnins *et al.*, 1988 and Sumiyoshi and Wargovich, 1990).

In the present study, the work has been divided into two parts: i) Clinical and ii) Experimental. An attempt has been made to gather information on alteration of various biochemical parameters mentioned earlier.

In clinical part, the changes in the activities of AChE, MAO and the levels of cortisol, SGOT, SGPT and LDH were estimated in anxiety neurotic, cancer breast and cancer liver patients before and after treatment. The alterations observed were compared with their respective normals.

In the experimental part, the alterations in the circulating activities of AChE, MAO, the levels of cortisol, SGOT, SGPT and RBC membrane osmotic fragility were assayed in experimental rats to evaluate the effect of restraint stress on DMBA indused cancer. Cancer was induced by single oral dose of DMBA (Roger *et al.*,

1990). The tissues (liver, kidney, heart, brain and spleen), the activities at AChE, GST, SOD and levels of reduced glutathione (total, free and protein bound) were also estimated in these rats.

The surgical treatments and modern therapies available for cancer have their own limitations and side effects. In Ayurvedic literature indigenous drugs, Garlic (Allium sativum *Liliaceae*) and Sage (Salvia officinalis *Labiatae*) are said to have some preventive and curative properties in cancer and other diseases. The preventive effects of these drugs on both initiation and promotion of DMBA induced carcinogenesis were studied. These drugs were selected because of their antioxidant properties and phenol/flavonoid contents, as flavonoid and phenols are reported to show anti-cancer properties. The possible mode of action of these drugs on DMBA carcinogenesis is evaluated in terms of biochemical parameters.

Material and Methods

CLINICAL :

Control : 70 apparently healthy and normal volunteers (35 males and 35 females, age ranging between 20-50 years) were selected and they served as normal controls. Total 12 ml of blood was collected from each volunteer in the fasting condition between 9 to 10 A.M. lying in supine position. Out of this 6 ml of blood was put in a heparinised tube, the plasma was separated by centrifugation and utilized for the estimation of cortisol (Mattingly, 1962), monoamine oxidase (McEwen and Cohen, 1963 and modified by McEven, 1971) and the erythrocytes were used for the estimation of acetylcholinesterase (Ellman *et al.*, 1961). Serum was separated from the rest of the blood and was used for the determination of the activities of glutamate oxaloacetate transaminase (Reitman and Frankel, 1957), glutamate pyruvate transaminase (Reitman and Frankel, 1957) and lactate dehydrogenase (Wroblewski and La Due, 1955). The details of the assay procedures employed in the present study are described later.

Anxiety neurosis : Fifteen patients suffering from anxiety neurosis were selected for this study from the psychiatric clinic of J.N. Medical College, Aligarh Muslim University, Aligarh. Detailed clinical history of each case was recorded and the diagnosis was confirmed by their symptoms, behaviour and case history. The blood was collected from each case twice, before the commencement of

treatment and after one month of treatment and was subjected for the estimations of cortisol, AChE, MAO, GOT, GPT and LDH.

Cancer Breast : This series included 15 patients of cancer of breast in which follow-up study was also done after one month of treatment (surgery,chemotherapy, radiotherapy). The diagnosis was confirmed by surgery and histopathological examination. Blood from each case was collected and subjected for the estimation of cortisol, MAO, AChE, GOT, GPT and LDH.

Cancer Liver : In this series 15 cases of cancer liver were included. These cases were admitted in the surgical ward of J.N. Medical College Hospital, Aligarh Muslim University. Detailed clinical history of each case was recorded and diagnosis was confirmed by surgical and histopathological examinations. Blood from each patient was collected at the time of admission and after 15 days of therapy and was subjected for the estimation of MAO, cortisol, AChE, GOT, GPT and LDH.

EXPERIMENTAL

The experimental models were studied to establish a correlation between clinical and experimental findings. The effect of stress on the development of DMBA induced mammary cancer and the prophylactic effects of indigenous drugs on initiation and promotion of DMBA induced carcinogenesis were evaluated on the

experimental models in terms of biochemical parameters as discussed below. Standardized methods of inducing mammary cancer (Rogers *et al.*, 1990) and restraint stress (Hasan *et al.*, 1980) were employed. After the termination of the experiment the rats were sacrificed, and the blood and tissue samples were collected according to the following procedures :

Collection of Plasma, Serum and Erythrocytes

After the termination of the experiment, the rats were anaesthetized by injecting sodium pentobarbitol (50mg/kg body weight, intraperitoneal). Heparin was used as anticoagulant for collection of plasma and RBC. Blood was centrifuged at 1000g for 10 minutes at 4°C, after centrifugation plasma/serum was collected carefully.

Serum was utilized for the estimations of LDH, GOT and GPT, while plasma for cortisol and MAO determinations.

Preparation of Erythrocytes :

To obtain erythrocytes, heparinized blood was centrifuged at 1000g for 5 minutes. The buffy coat was removed, the red blood cells were suspended in 10 volumes of cold 0.1M phosphate buffered saline pH 7.4. Cells were washed in this manner 5 times to remove plasma proteins. During each wash a small portion of the top of the cell pellet was removed to ensure maximal removal of leukocytes. The activity of acetylcholinestrase and osmotic fragility were estimated in the erythrocytes.

Collection of Tissues :

Brain, heart, liver, kidney and spleen were dissected out immediately from each rat after the injection of sodium pentobarbital (50mg/kg body weight, i.p.). The tissues were washed with chilled normal saline. The tissue samples were subjected for the estimation of AChE, GST, SOD and glutathione. The homogenization, was carried out as follows :

Preparation of tissue homogenates:

The homogenate of various rat tissues (hear, liver, kidney, brain and spleen) were prepared as follows:

1) For AChE activity: 20 mg tissues/ 1ml 0.1M phosphate buffer pH 8.0.

ii) For GST activity: 10% (w/v) in 0.2M phosphate buffer pH 6.5.

iii) For GSH, and SOD: 10% (w/v) in chilled 0.15 M KCL.

Quantitive Estimation of Proteins :

The procedure described by Lowry *et al.*, (1951) was followed. Suitable aliquot of the protein samples (Washed erythrocytes, plasma and clear supernatant of different tissues) were diluted to 1.0 ml with distilled water. To this a 5.0ml of freshly prepared copper reagent was added (copper reagent was prepared by mixing 0.5% copper sulphate, 1% (w/v) sodium potassium tartarate, 2% (w/v) sodium carbonate in 0.1N NaOH in 1:50 ratio). After incubation for 10 minutes at room temperature, 0.5ml of 1N folin's reagent was added.

The contents were rapidly mixed and colour intensity of the reaction was read after 30 minutes against a reagent blank at 660 nm. The concentration of proteins in different samples were determined using a standard curve with BSA.

1. Restraint stress and Dimethylbenz(a)anthracene induced mammary cancer :

Total 140 female albino rats (Spraque-Dawley strain), weighing around 40g±5g of same age (45-55 days) were selected. Before experimentation, the rats were deprived of food for 12 hours and water was supplied *ad libitum*. Through out the experiment the animals were supplied with food and water *ad libitum*. The animals were divided into following groups:

Group I (20 rats) : These rats were kept at laboratory conditions and were given food and water *ad libitum* till the termination of the experiment. These animals received 1 ml of sesame oil once instead of DMBA and served as controls.

Group II (100 rats): This group of rats were kept at laboratory conditions for 129 days (so that the age of the stress treated and DMBA infused rats remains same.) and then exposed to restraint stress by standardized method (Hasan *et al.*, 1980). The rats were divided into five groups of 20 rats each and they were immobilized in individual cages of their size for 6, 12, 18, 24 and 30 hours at laboratory conditions (Figs. 7 & 8).

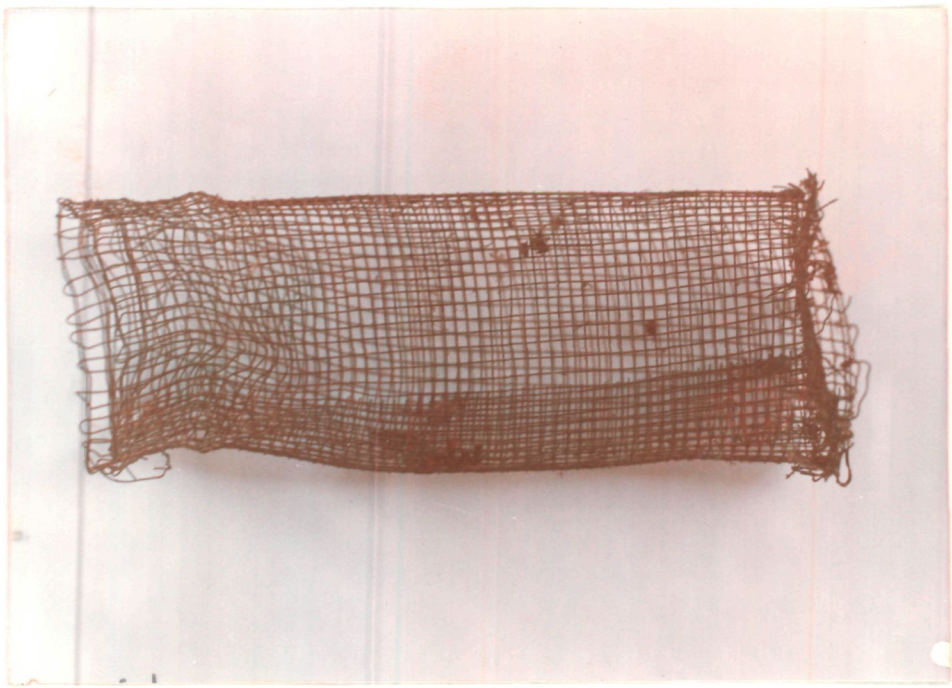


Fig. 7 : Cage used for immobilization stress treatment.

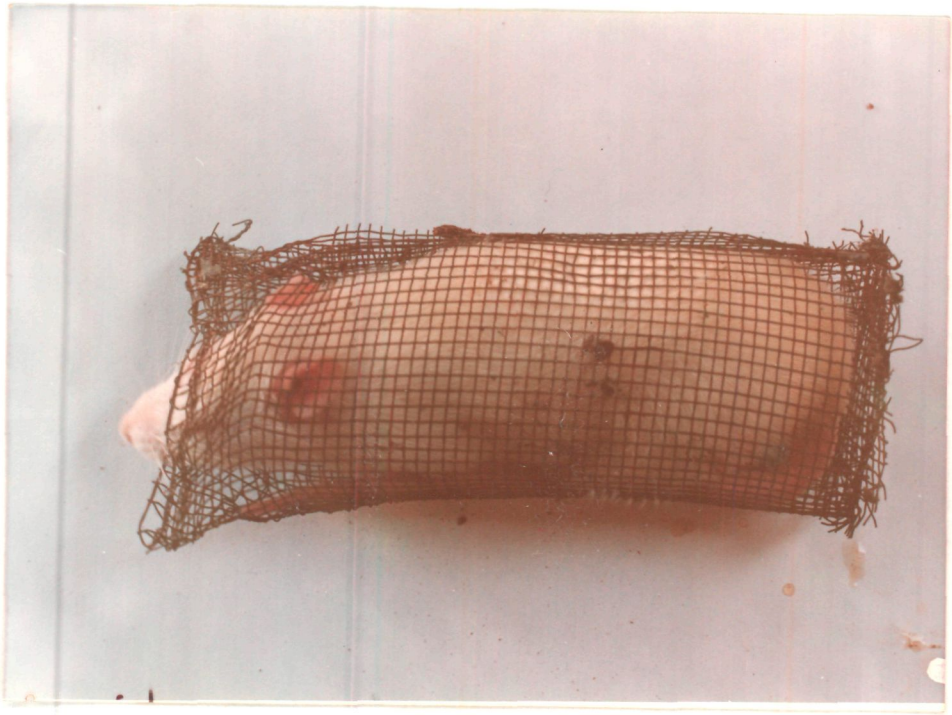


Fig. 8 : Rat immobilized in a body size cage.

Group III (20 rats) : These rats were treated with (DMBA) dimethylbenz(a) anthracene (Sigma Chemical Co.) by gavage (30mg/ kg body weight dissolved in 1ml sesame oil). The standardized method of tumor induction (Rogers *et al.*, 1990) by a single dose of DMBA administration was followed. The weight, palpability and dietary intake of all these rats were monitored every ten days after 5 weeks of DMBA administration. The rats were sacrificed after 130 days of DMBA administration.

2. The effect of stress on DMBA induced cancer:

The effect of stress (both pre and post) was seen on the DMBA induced mammary carcinogenesis in female rats in terms of above mentioned biochemical parameters. For this study, the animals were divided into following groups :

A. Effect of Pre-stress treatment :

Group-I(A) : (20 Rats): These rats were exposed to 24 hours restraint stress prior to the oral administration of DMBA (30mg/kg body weight dissolved in 1ml sesame oil). The rats were monitored for their body weight, intake of diet and palpability every ten days after 5 weeks of DMBA administration. This group served as pre-stress treated rats.

Group-II(A): (20 rats): These rats were infused with DMBA (30 mg/ kg body weight dissolved in 1ml sesame oil) alongwith the group I(A) and served as control group (cancerous without stress) for the

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To evaluate the effect of stress on DMBA induced carcinogenesis, the rats (S-D) were divided into following groups

I Control rats (20)	II Stress treated rats (20)	III DMBA infused rats (60)	IV Pre-stress treated DMBA infused rats (20)	V DMBA infused post-stress treated rats (20)
The rats were kept at laboratory conditions and given single dose off 1ml sesame oil by gavage.	Rats were exposed to 24 hours immobilization after 129th day of commencement of the experiment.	Rats were given DMBA (30 mg/kg body weight dissolved in 1 ml sesame oil) by gavage.	On the Ist day, the rats were exposed to24 hours immobilization stress and then were given DMBA by gavage (single dose, 30 mg/kg body weight, in 1 ml sesame oil)	On the Ist day, the rats were infused with DMBA (30 mg/kg body weight, single dose, in 1 ml sesame oil) and then they were exposed to 24 hours immobilization.

All these rats were then fed food and water *ad libitum* throughout the experiment

Sacrificed at 130th day.	Sacrificed at 130th day.	Divided into three groups of 20 rats each and sacrificed on 100th, 110th (controls for IV and V group) and 130th day.	Sacrificed on 110th day of DMBA infusion.	Sacrificed on 100th day of DMBA infusion.

No. in paranthesis indicate the number of rats.

pre-stress DMBA induced cancerous rats, group I(A) above. The weight, palpability etc. was monitored for this group too as mentioned earlier. Both these groups were sacrificed after 110 days of DMBA treatment due to the deteriorating conditions of group I(A) rats. Blood and tissue samples were collected and were subjected for the assay of cortisol, MAO, GOT, GPT, LDH, SOD, GST, AChE and reduced glutathione (total, free and protein bound GSH).

B. Effect of Post-stress treatment :

Group-I(B):(20 Rats): Each rat of this group received DMBA (30mg/ kg body weight dissolved in 1ml of sesame oil) by gavage and then exposed to restraint stress (immobilization) for 24 hours as described earlier. The animals were supplied with food and water *ad libitum* and their weight, palpability and food intake was checked every ten day after 5 weeks of DMBA treatment. This group served as post-stress treated rats.

Group-II(B): (20 Rats): Each rat received DMBA (as mentioned above) by gavage and served as cancerous control group for group I(B) (Post-stress treated). The rats of both the above groups had to be sacrificed after 100 days of commencement of the experiment due to the fast deteriorating condition of group I(B).

Effect of indigenous drugs, Garlic (Allium *sativum*) and Sage (Salvia *officinalis*) on DMBA induced cancer in rats:

140 female Spraque-Dawley rats weighing around 40 $\pm$5gm and 40 days $\pm$5 days old were included in this series. These rats were

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The rats (S-D) were divided into four different groups to evaluate the prophylactic and curative effects of indigenous drugs.

I Control rats (20)	II DMBA infused (Cancerous) rats (20)	III Pre-drugs treatment (40)	IV Post-drugs treatment (40)
The rats were kept at laboratory conditions and given 1 ml saline for 20 days, by gavage.	DMBA (30 mg/kg body weight) was given once orally, dissolved in 1 ml sesame oil.	Rats were divided into two groups of 20 rats each. One group received garlic (1 ml, 25 gm/100 ml), while another received salvia (25 gm/100 ml) for 20 days prior to DMBA (30 mg/kg body weight, in 1 ml sasame oil) infusion.	40 rats were given DMBA orally in 1 ml sesame oil (30 mg/kg body weight). Then were divided into two groups of 20 rats each. One group received garlic (1 ml for 20 days of 25 g/100 ml) while another received salvia (1 ml for 20 days, 25 g/100 ml).

Food and water supplied to all these rats *ad Libitum* and were sacrificed on 130th day of experimentation.

divided into following groups. Fresh garlic was bought from the local market while salvia was procured from **Jordan** (Middle East):(Fig. 9).

Group I (20 rats): In this group, each rat received 1ml of 0.1% saline daily for 20 days. They were kept at laboratory conditions and were given food and water *ad libitum* till the termination of the experiment (served as normal controls).

Group II : This group included 60 rats. These rats were further divided into three subgroups of 20 rats each to assess the preventive role of salvia and garlic (indigenous drugs) on the initiation of carcinogenic effect of dimethylbenz(a)anthracene(DMBA).

Sub-group II(A) : One ml of saline given orally once a day for twenty days prior to DMBA infusion and these served as controls (cancer control) for subgroups II(B) and II(C).

Sub-group II(B) : One ml of garlic extract (25gms/100ml of 0.1% aline) was administered orally once a day for twenty days prior to cancer induction by DMBA infusion.

Sub-group II (C) : One ml of salvia extract (25gm/100ml in 0.1% saline) was given orally for 20 days prior to cancer induction. Thereafter, the treatment was stopped 24 hours prior to the DMBA oral infusion (30mg/kg body weight in 1ml sesame oil).

Group III: The remaining 60 rats were included in this group and were again divided into three different subgroups of 20 rats each, to determine the preventive effect of salvia and garlic on the promotion of DMBA induced cancer.

Sub-group III (A): One ml of 0.1% saline was given orally for 20 days after DMBA infusion and these served as cancerous control for subgroups III(B) and III(C).

Sub-group III(B): Garlic (same dose as given to sub-group II(B) animals) was administered orally once a day for 20 days after 24 hours of an oral infusion of DMBA (30mg/kg body weight).

Group III (C) : Salvia solution (in the same dose as given to sub-group II(C) animals) was given orally once a day for 20 days after 24 hours of a single dose oral infusion of DMBA.

All the six groups of animals were sacrificed after 130 days of commencement of experiment. Blood and tissue samples were collected from each rat. Blood samples were used for the assay of AChE, MAO, cortisol, SGOT, SGPT, LDH and osmotic fragility.The tissues from brain, heart, liver, kidney and spleen were subjected for the estimation of AChE, GST and SOD. The levels of total, free and protein bound GSH were also estimated in the above tissues. The biochemical procedures are discussed later.

Throughout the study students 't' test was used for statistical analysis.

The estimations of Biochemical Parameters :

Acetylcholinestrase (AChE) :

(Ellman *et al.*, 1961)

Principle :

The enzyme catalyses the hydrolysis of substrate (acetyl-thiocholine iodide) into thiocholine and acetate. the thiocholine thus formed reacts with dithiobisnitrobenzoate to give yellow colour. The absorbance of this yellow colour is measured in the spectro-photometer at 412 nm.

$$\text{Acetylthiocholine iodide} \xrightarrow{\text{AChE}} \text{Thiocholine} + \text{Acetate}$$

$$\text{Thiocholine+dithiobisnitrobenzoic acid} \longrightarrow \text{yellow colour (absorbed at 412nm).}$$

Reaction:

$$\underset{\text{(thiocholine iodide)}}{(CH_3)_3NCH_2CH_2SCOCH_3} \xrightarrow{\text{AChE}} \underset{\text{(thiocholine)}}{(CH_3)_3\ N^+\text{-}CH_2\text{-}CH_2\text{-}S^-} + \underset{\text{(acetate)}}{CH_3COO^- + 2H^+}$$

$$\underset{\text{(acetate)}}{(CH_3)_3N^+\text{-}CH_2\text{-}CH_2\text{-}S} + \underset{\text{(DTNB)}}{R\text{-}S\text{-}S\text{-}R} \longrightarrow \underset{\text{(yellow colour)}}{(CH_3)_3NCH_2SSR} + RS$$

Reagent :

i. 0.1M Phosphate buffer pH 8.0 and 0.1M, phosphate buffer pH 7.0.

ii. Substrate;Acetylthiocholine iodide 0.075M (21.67mg/ml) stable for 10-15 days if kept refrigerated.

iii. Sodium bicarbonate AR.

iv. Reagent: Dithiobisnitorbenzoic acid (DTNB). 0.01M of the 5,5' dithiobis-2-nitrobenzoic acid 39.6 mg were dissovled in 10ml of pH 7.0 phosphate buffer (0.1M) and 15 mg of sodium

bicarbonate were added. The reagent was made in buffer pH 7.0 in which it was more stable than in that of pH 8.0.

v. Working blood solution: 10 µl (0.01ml) of washed erythrocytes were suspended in 12.0 ml of 0.1M phosphate buffer of pH 8.0 (dilution, 1:1200). Since the AChE is bound on the cell membrane of RBC, hemolysis was not necessary.

Method for RBC :

Exactly 3.0ml of the suspension of washed RBC (diluted 1:1200) were pipetted into a cuvette, 25 µl of DTNB reagent was added. The cuvette was then placed into the spectrophotometer and its slit was adjusted so that the absorbance (at 412nm) of the suspension in the cuvette was zero. 20µl of the substrate was added to this cuvette. Changes in the absorbance at 412nm were recorded for at least 6 minutes period. Protein was estimated in the rest 3ml suspension of RBC by the method of Lowry *et al.* (1951).

Calculations:

Moles substrates hydrolyzed/min/mg protein

$$= 0.0882 \quad \frac{600}{13.600} \times \frac{A}{\text{Protein concentration(mg)}}$$

$$= (8.82).10^{-5} \frac{A}{\text{Protein concentration(mg)}}$$

where 8.82×10^{-5} = factor for dilution and extinction coefficient.

Method for Tissues:

The tissues were homogenized (20mg/ml) in phosphate buffer (pH 8.0, 0.1M). The muscular tissues were minced considerably before homogenizing. 0.4ml aliquot of this homogenate was added

to a cuvette containing 2.6ml of phosphate buffer (pH 8.0, 0.1M). To this, 100µl of DTNB reagent was added. The absorption was set to zero at 412 nm and 20 µl of the substrate was added. Changes in absorbance were recorded and the change in absorbance per minute was calculated. The rates were calculated as follows :

$$R = \frac{A}{1.36\ (104)} \times \frac{1}{(400/3120)Co}$$

$$= 5.74\ (10^{-5})\ A/Co$$

where R=rate in moles substrates hydrolysed per minute per gm. of tissues.

A = Change in absorbance per min.
Co = Original concentration of tissues (mg/ml).

Plasma Monoamine Oxidase (MAO) :
(McEven and Cohen, 1963 modified by McEven, 1971):

Principle: The measurement of MAO activity depends on the conversion of amine to aldehyde. The substrate benzylamine is converted into benzaldehyde which is extracted in the organic layer (cyclohexane) and is measured spectrophotometrically at 242nm.

$$RCH_2NH_2 \xrightarrow{MAO} RCHO+NH_3.$$

Reagents:

i. Phosphate buffer 0.2M, pH 7.2
ii. Benzylamine 8mM in 0.2M phosphate buffer pH 7.2
iii. Perchloric acid 60%
iv. Cyclohexane AR

Method : To 1.0ml of plasma, 1.25ml of phosphate buffer and 0.25ml of substrate (benzylamine) were added. This was incubated in

Dubnoff's metabolic shaker for 3 hours. The reaction was stopped by the addition of 0.25ml of 60% PCA. A control tube was also run alongwith it, in which substrate was added after stopping the reaction. The mixture of both the control and the experimental tubes were properly shaken and 2.5 ml of cyclohexane was added (for aldehyde extraction). It was stirred and allowed to stand for 15 minutes at room temperature. The emulsion was centrifuged and the cyclohexane layer was separated (This step was repeated with 2.5ml of cyclohexane). The absorbance of the cyclohexane extracts were measured at 242nm in silica cells against plain cyclohexane.

Contents of the Tubes:

Control Tube		Experimental Tube	
Plasma	1.0ml	plasma	1.0ml
Buffer	1.25ml	Buffer	1.25ml
PCA	0.25ml	Benzylamine	0.25ml
Benzylamine	0.25ml	PCA	0.25ml

Calculations:

The difference of 0.01 in optical density in between control and experimental samples using 1ml plasma is equivalent to one provisional unit (P.U.). The results were expressed in terms of P.U. (provisional unit)/ 1ml of plasma.

Plasma Cortisol :
(Mattingly, 1962)

Principle :

A very high flourescence is given by cortisol at either a very

high or a very low pH. Thus, cortisol is extracted and the fluorescence is measured after dissolving it in a strongly acidic medium.

Reagents :

i. Cortisol standard 1 ug/1ml.
ii. Dichloromethane AR
iii. Fluorescence reagent : volumes of H_2SO_4 is mixed in cold with 3 volumes of ethanol.

Method :

Two ml each of plasma, water and standard cortisol solution (1 µg/ml) were pipetted in glass stoppered extraction tubes which served as unknown tube, blank and standard tubes respectively. 15ml of dichloromethane was added in each tube, and the tubes were rotated gently from one end to another for 20 minutes. The phases then were allowed to separate and the supernatent aqueous layer was discarded. The organic layer was transferred to a tube containing 5 ml of the "fluorescence reagent" and the contents were shaken for 20 minutes. After 20 minutes the fluorecence of the aqueous layer was measured at excitation = 470 and emission =530nm.

The concentration of cortisol was expressed in terms of µg/ 100ml.

Glutathione :
(Ellman, 1959, modified by Sedlack and Lindsay, 1968).

Principle:

The determination of total, free and protein bound sulfhydryl groups were based on the reduction of 5-5', dithiobis-2-nitrobenzoic acid (DTNB) by -SH group of glutathione (GSH) in alkaline medium

to reduced one mole of 2-nitro 5-mercaptobenzoic acid per mole of -SH group. The reaction was measured at 412nm.

Reagents :

i. Standard: Glutathione (reduced) $2x10^{-3}M$.
ii. 0.02M EDTA.
iii. 0.15 M KCL.
iv. 0.2M Tris-EDTA buffer, pH 8.2
v. 0.4M Tris-EDTA buffer, pH 8.9.
vi. 0.01M DTNB (dissolved in absolute alcohol).
vii. 10% TCA.

T-5493

Procedure :

Total reduced glutathione (GSH-total) : Various tissues (heart, brain, liver, kidney and spleen) of the rats were homogenized in chilled 0.15M KCL and the volume of the homogenate was adjusted to 10% (w/v). To 0.1ml of homogenate, 1.5ml of 0.2M Tris-EDTA buffer (pH 8.2) and 0.1ml DTNB were added. The solution was mixed properly and the volume was made upto 10 ml with 8.3 ml absolute alcohol. The solution was centrifuged at 6000g for 5 minutes in cold. The precipate was discarded and the absorbance of the clear supernatant was read at 412nm. A calibration curve with different concentrations of GSH (200-1600 µmoles) was obtained according to the same procedure as described above. The standard curve was used for the calculation of total -SH group present in the samples and the results were expressed as µmole of -SH group/gm tissues.

Non-Protein bound glutathione (GSH-free) :

1.0ml of tissues homogenate (10%) prepared in 0.1M KCl was deproteinized by adding 1.0ml of 10% TCA. The tubes were kept at 4°C for 20 minutes and then centrifuged at 6000g for 5 minutes. 0.5ml of the clear supernatant was mixed with 0.5ml water, 2ml of 0.4M Tris-EDTA buffer pH 8.9 and 0.1ml of 0.01M DTNB (prepared in absolute methanol) with proper stiring. The absorbance was read at 412nm within 30 minutes of the addition of DTNB. For the calibration curve, different concentations of GSH (200-1600 µmoles) were also run by the same procedure as described above.

Protein bound reduced glutatione (GSH-protein bound) :

Protein bound sulfhydryl groups were determined by subtracting free GSH from total GSH, as described by Sedlak and Lindsay (1968).

Protein bound GSH group = Total GSH group–Free GSH group.

Glutathione-S-Transferase (GST) :
(Habig *et al.*, 1974).

Principle:

The enzyme activity is measured by following the increase in absorbance at 340nm of CDNB-GSH conjugate generated as a result of GST catalysis between glutathione and 1-Chloro-2, 4-dinitro-benzene (CDNB).

$$\text{CDNB} + \text{GSH} \xrightarrow{\text{GST}} \text{CDNB-GSH Conjugate.}$$

Reagents :

i. 1.0mM Glutathione (Reduced).
ii. 0.2M phosphate buffer pH 6.5
iii. 1.0mM CDNB (prepared in acetone).

Procedure :

Different tissues (brain, heart, liver, kidney and spleen) of rats were homogenized in chilled 0.2M phosphate buffer pH 6.5 (10% w/ v) and centrifuged in cold for 15 minutes at 1500g. To 0.1ml of tissue supernatant, 2.7 ml 1mM glutathione solution (in 0.2M phosphate buffer pH 6.5) and 0.2 ml of 1.0mM CDNB were mixed. The change in absorbance at 340 nm was recorded at room temperature against blank (containing all the reagents except the enzymes). Protein content in enzyme source was also determined by the method of Lowry et al. (1951).

Calculation :

The values were calculated on the basis of molar extinction coefficient of CDNB ($9.6 \times 10^3 M^{-1} Cm^{-1}$) and specific activity of enzyme was expressed in μmoles of GSH-CDNB conjugate formed per minute per mg protein.

$$\text{GST activity} = \frac{\text{O.D. x 625 (Factor)}}{\text{protein concentration (mg).}}$$

$$= \text{Units/1mg protein}$$

O.D. = Change of optical density per minute.

Where 625 is a factor for dilution and extinction coefficient.

Superoxide Dismutase (SOD) :
(Markland & Markland, 1974)

Principle :

The SOD activity determination is based on the conversion of $O^{\cdot}_2$(the products of pyrogallol autoxidation) to H_2O_2, which is measured at 420nm.

Reaction :

$$\text{Pyrogallol} + O_2 \xrightarrow{\text{Autoxidation}} \text{Oxidation product} + O^{\cdot}_2$$

$$2O^{\cdot}_2 + 2H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

Reagents :

i. 0.05M Tris succinate buffer (pH 8.2).
ii. 0.05M Succinic acid solution.
iii. 8mM Pyrogallol solution (freshly prepared).

Procedures :

2.85ml of 0.05M Tris-succinate buffer pH 8.2 and 0.05ml of tissues supernatant, mixed and incubated at 25°C for 20 minutes. The reaction was started by adding 0.1ml pyrogallol solution. Changes in absorbance per minute were immediately recorded for an initial period of 3 minutes at 420nm. A reference set, consisting of 0.05 ml distilled water instead of the homogenate sample was also run similarly.

Calculation :

$$\text{SOD Activity} = \frac{(\Delta A/\text{min.ref} - \Delta A/\text{min. Sample}) \times 30}{\Delta A/\text{min.ref./2} \times 0.05 \times 10}$$

= Units/mg tissue.

where $\Delta A/\text{min. ref.}$ = change of absorbance per minute in reference set.

and $\Delta A/\text{min. sample}$ = Change of absorbance per minute in sample set.

Activity Unit : One unit of enzyme is defined as the amount of enzyme which causes a 50% inhibition of pyrogallol autoxidation under assay conditions.

Transaminases :

(Reitman and Frankel, 1957):

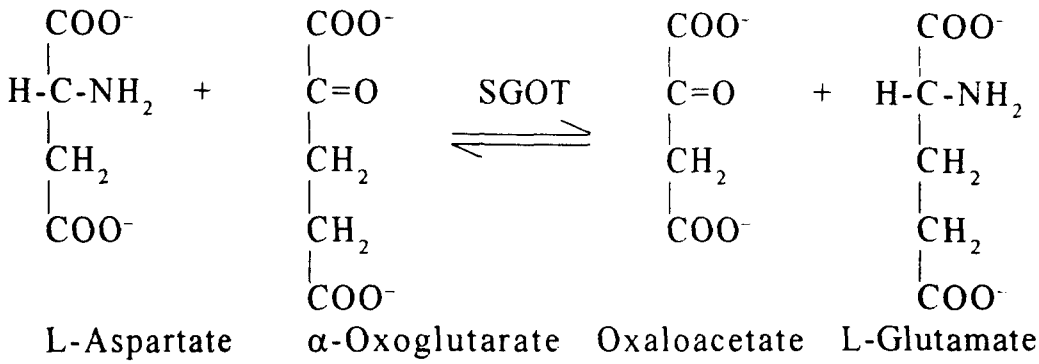
Glutamate oxalacetate (GOT):(EC.2.6.1.1.) and glutamate pyruvate transaminase (GPT):(EC.2.6.1.2.)

Principle :

The transamination involves the transfer of α-amino group from α-amino acid to α-oxoacids, usually 2-oxoglutarate. The major 2-oxoglutarate linked transaminases are aspartate transaminases (GOT) and alanine transaminases (GPT).

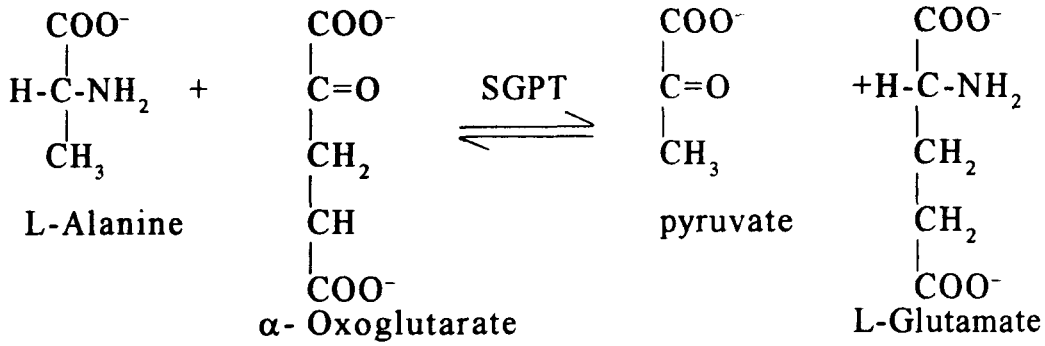
The reactions :

(A) For GOT:



L-Aspartate α-Oxoglutarate Oxaloacetate L-Glutamate

(B) For GPT :



The pyruvate formed by GOT transamination, reacts with 2,4-Dinitrophenyl hydrazine (DNPH) to give a brown coloured hydrazone, which is measured colorimetrically at 510 nm.

The oxaloacetate is decarboxylated spontaneously to pyruvate by GPT, which is again measured by hydrazone formation.

Reagents:

i. Phosphate buffer 0.1M pH 7.4.
ii. 200 mM L-aspartic acid.
iii. 200 mM L-alanine.
iv. 2 mM α-ketoglutarate.
v. Stock pyruvate standard: (20 mM) in 0.1M phosphate buffer pH 7.4.
vi. Working pyruvate standard: (4 mM) freshly prepared from the above stock.
vii. 1 mM 2,4-dinitrophenyl hydrazine.
viii. 0.4 N. sodium hydroxide.

Methods :

Glutamate Oxaloacetate Transaminases : (GOT)

Test (T): 0.5ml of substrate (aspartic acid; α-Ketoglutarate) were warmed in a water bath at 37°C for 3 minutes prior to the addition

of 0.1ml of serum. Mixed gently and incubated again for 60 minutes exactly. The tubes were removed from the water bath and 0.5ml of DNPH solution were added and Mixed well.

Three other test tubes (control, standard and blank) were prepared as follows :

	Tube I Contol (C)	Tube II Standard (S)	Tube III Blank (B)
Substrates (aspartic acid; α-Ketoglutarate)	0.5ml	0.4ml	0.5ml
Working pyruvate	--	0.1ml	--
Water	--	0.1ml	0.1ml
DNPH	0.5ml	0.5ml	0.5ml
Serum	0.1ml	--	--

In all the above tubes, the DNPH was allowed to react for 20 minutes at room temperature, then 5ml of 0.4 N sodium hydroxyde were added, mixed well and the reactions were allowed to proceed for another 10 minutes. The optical density of all the tubes were measured at 510nm.

The pyruvate formed per minute per litre of serum

$$= \frac{T\text{-}C}{S\text{-}B} \times 0.4 \times 1/60 \times \frac{1000}{0.1}$$

$$= T\text{-}C/S\text{-}B \times 67 \ \mu moles.$$

The calculated pyruvate was converted into international units per litre.

2. Glutamate Pyruvate Transaminases : (GPT)

The procedure is same as in GOT. While L-alanine was used as substrate instead of L-aspartate and the incubation time in (T) test was reduced to 30 minutes (instead of that of 1 hour for GOT).

$$\text{The pyruvate formed per minute per litre of serum} = \frac{\text{T-C}}{\text{S-B}} \times 0.4 \times 1/30 \times \frac{1000}{0.1}$$

$$= \text{T-C/S-B} \times 133 \ \mu\text{moles}.$$

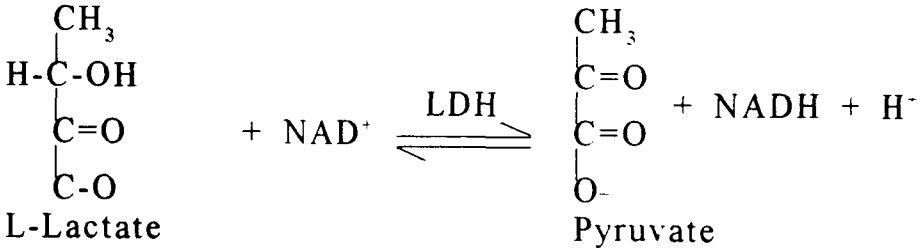
The calculated pyruvate was converted into international units per litre.

Lactate dehydrogenase (LDH) :
(Wroblewski and La Due, 1955).

Principle :

Pyruvate is reduced to lactate at pH 7.4, 37°C in the presence of LDH. The progress of the accompanying oxidation of NADH to NAD^+ is monitored continously, by measuring the rate of decrease of absorbance at 340 nm spectrophotometrically .

The reaction:



Reagents:

i. 0.1M phosphate buffer pH 7.4.
ii. NADH (2.5mg/ml 0.1M phosphate buffer pH 7.4).
iii. Sodium pyruvate (2.5mg/ml) in 0.1M phosphate buffer pH 7.4).

Procedure :

To 2.4 ml of 0.1M phosphate buffer (pH 7.4), 0.1ml of non-hemolyzed serum and 0.1ml NADH were added. The reaction was allowed to proceed at room temperature for 20 minutes. then 0.1ml of sodium pyruvate was added, and the change in optical density at every 30 second interval was recorded at 340 nm for 5 minutes.

The optical density was measured against blank (consisting of all reagents except sodium pyruvate) giving 100% transmittance.

Calculation :

Decrease in O.D. of test solution in 5 minutes x 1000 = Units LDH activity per ml serum.

Osmotic fragility of erythrocytes :
(Orcutt *et al.*, 1995)

The erythrocytes of blood collected after centrifugation were washed five times with normal saline (0.9%) (The leukocytes and the buffy coat were removed after centrifugation). The washed RBCs were diluted in 0.1M phosphate buffer pH 8.0 (1:1200) and were suspended in different concentrations (0.1% to 0.9%) of saline. This leads to the release of hemoglobin of erythrocytes. The extent of fragility (hemolysis) of RBC membranes in the presence of various concentrations of saline was measured in terms of quantity of hemoglobin released. The release of hemoglobin was monitored colorimetrically at 545 nm (Orcutt *et al.*, 1995).

Procedure :

To 0.2 ml of washed and diluted (1:1200) suspension of RBC, 2.3ml of 0.1M phosphate buffer pH 8.0 containing different concentrations of saline (0.1% to 0.9%) was added (in different test tubes) and incubated at room temperature for 30 minutes, then was monitored at 545 against 0.1M phosphate buffer pH 8.0 (as blank).

The pH 8.0 of phosphate buffer was selected because at this pH, the hemoglobin of rat showed maximum solubility (Luque *et al.*, 1992).

Results

CLINICAL

1. **Normal levels of males and females (35 males + 35 females)**

The normal circulating levels of cortisol, activities of AChE and MAO were higher in females than in males, while the serum levels of GOT, GPT and LDH were higher in males than in females, though the differences were not significant (Table - 1)

2. **Anxiety neurosis (15 patients) :**

In the patients of anxiety neurosis, the circulating levels of cortisol, GOT, GPT and LDH were significantly raised ($P<0.001$) and the activities of RBC AChE and plasma MAO were significantly decreased ($P<0.001$) in comparison to controls.

The biochemical parameters reverted towards their corresponding normal values after one month of chemotherapy in these patients, and were less significantly altered ($P<0.05$) from normals (Table - 2). A slight but insignificant increase in the levels of SGOT and SGPT was observed after treatment (Tables 2 and 3, Figs. 10 and 11).

3. **Cancer Breast (15 patients) :**

The circulating cortisol, GOT, GPT and LDH levels were significantly raised ($P<0.001$) while RBC AChE and plasma MAO activities were decreased in the patients of breast cancer. After one month of surgical treatment (12 patients), these levels reverted towards control values except the levels of GOT and GPT which showed a slight further increase(Tables 4 and 5, Figs. 12 and 13).

Table - 1

Sexwise difference in the circulating levels of AChE, MAO, Cortisol, LDH, GOT and GPT in normal human beings

(Mean ± S.E.M.)

	RBC AChE (µ moles/min/ mg Proteinx10^{-5}	Plasma MAO (PU/ml)	Plasma Cortisol (µg%)	SGOT (IU/ml)	SGPT (IU/ml)	LDH (U/ml)
Male (35)	5.640 ±0.440	10.934 ±0.214	16.680 ±1.020	29.300 ±4.450	24.730 ±2.850	187.30 ±11.60
Female (35)	5.660 ±0.308	12.084 ±1.014	17.230 ±1.220	25.900 ±3.500	19.86 ±2.195	178.54 ±12.61
Normal of either sex (70)	5.648 ±0.373	11.810 ±0.800	16.943 ±0.092	28.101 ±3.920	22.33 ±2.473	183.17 ±11.97

Table - 2

Changes in the circulating levels of AChE, MAO and Cortisol in Anxiety neurotic patients before and after treatment

(Mean ± S.E.M.)

	Normal (35)	Anxiety neurotic Patients	
		before treatment (15)	after treatment (15)
RBCs AChE µmole/min/mg protein x 10^{-5}	5.640 ±0.440	2.760[a] ±0.142	3.904[b] ±0.144
Plasma MAO PU/ml	10.934 ±0.214	5.701[a] ±0.105	7.450[b] ±0.110
Plasma Cortisol µg%	16.680 ±1.020	25.620[a] ±1.861	19.926[a] ±1.608

* The numbers in paranthesis indicate the number of patients.

* a $p<0.001$, b $p<0.01$, c $p<0.05$ in comparison to normals.

Changes in circulating levels of AChE, MAO and Cortisol in Anxiety neurotic patients before and after treatment.

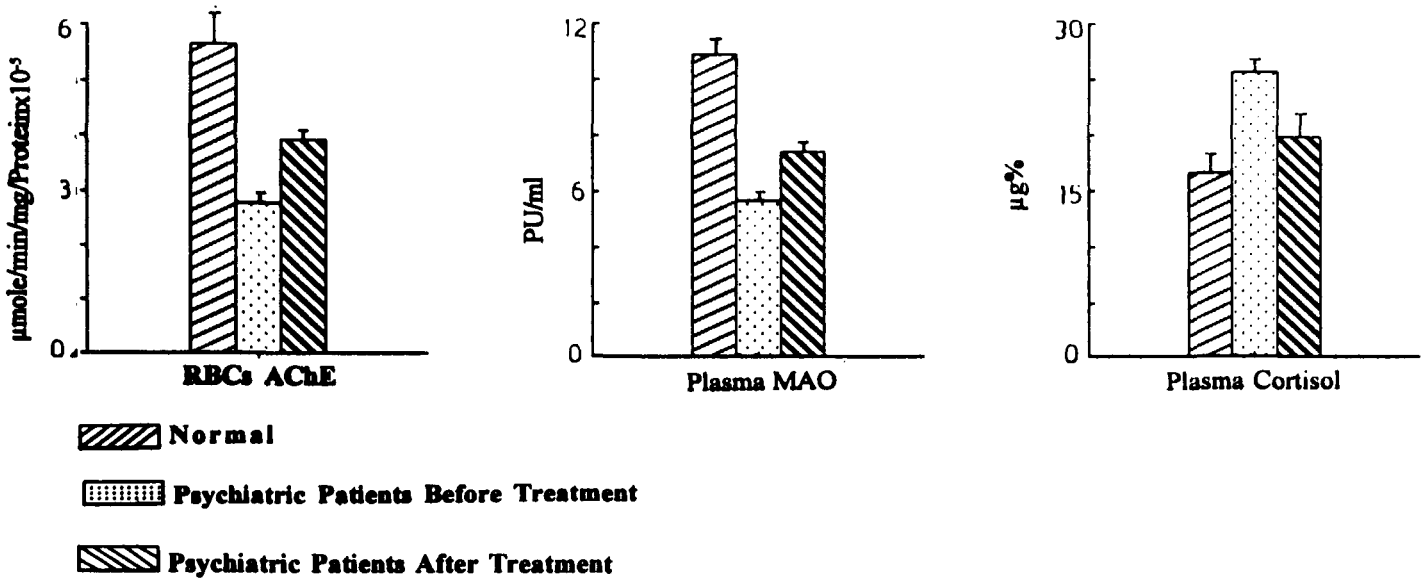


Fig. 10

Table - 3

The levels of serum GOT, GPT and LDH in anxiety neurotic patients before and after treatment

(Mean ± S.E.M.)

	Normal (35)	Anxiety neurotic patients	
		before treatment (15)	after treatment (15)
GOT IU/ml	27.30 ±4.45	53.36[a] ±4.87	57.41[a] ±5.21
GPT IU/ml	24.73 ±2.85	44.60[a] ±3.64	49.12[a] ±2.85
LDH U/ml	187.20 ±11.60	218.04[b] ±17.74	198.10[c] ±11.21

* The numbers in paranthesis indicate the number of patients.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ in comparison to normals.

The levels of serum GOT, GPT and LDH in Anxiety neurotic patients before and after treatment.

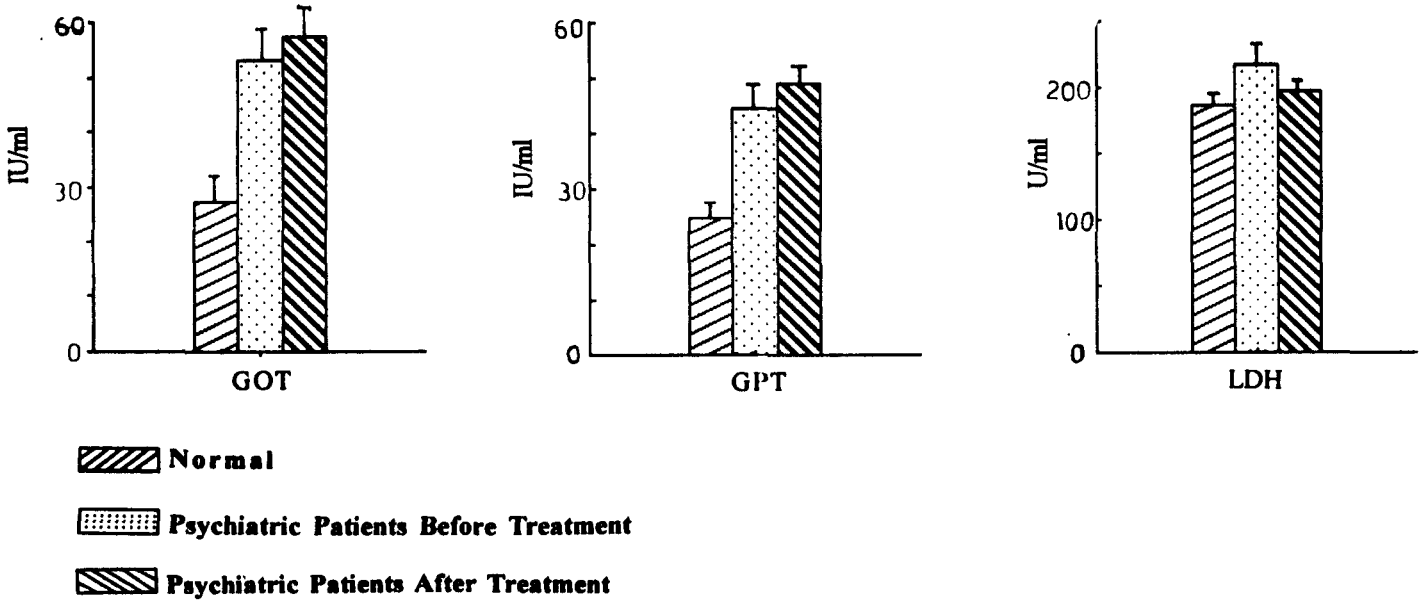


Fig.11

Table - 4

Changes in the circulating levels of AChE, MAO and Cortisol in breast cancer patients before and after treatment

(Mean ± S.E.M.)

	Normal (35)	Breast Cancer Patients	
		before treatment (15)	after treatment (12)
RBCs AChE μ mole/min/mg prot.x10^{-5}	5.666 ±0.308	3.034[a] ±0.280	3.396[a] ±0.160
Plasma MAO PU/ml	12.084 ±1.014	5.527[a] ±0.210	6.350[a] ±0.350
Plasma Cortisol μg%	17.230 ±1.220	33.750[a] ±0.950	25.090[a] ±0.920

* The number in paranthesis indicate the number of patients.

* a $p<0.001$, b $p<0.01$, c $p<0.05$ in comparison to normals.

Changes in the circulating levels of AChE, MAO and Cortisol in Cancer breast patients before and after treatment.

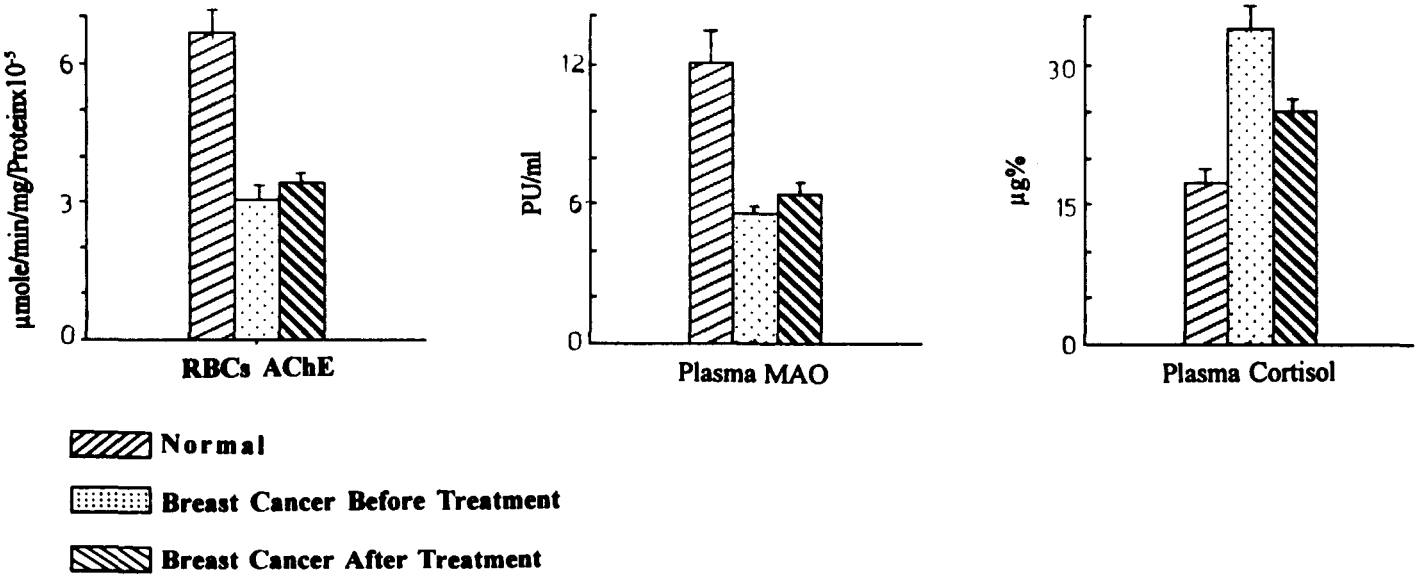


Fig.12

Table - 5

The levels of serum GOT, GPT and LDH in cancer Breast patients before and after treatment

(Mean ± S.E.M.)

	Normal (35)	Cancer Breast Patients	
		before treatment (15)	after treatment (12)
GOT IU/ml	25.90 ±3.50	78.49[a] ±5.05	85.30[a] ±7.45
GPT IU/ml	19.86 ±2.20	63.95[a] ±4.93	78.60[a] ±4.80
LDH U/ml	178.54 ±12.61	243.00[a] ±13.40	210.11[b] ±16.60

* The numbers in paranthesis indicate the number of patients.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ in comparison to normals.

The levels of serum GOT, GPT and LDH in Cancer breast patients before and after treatment.

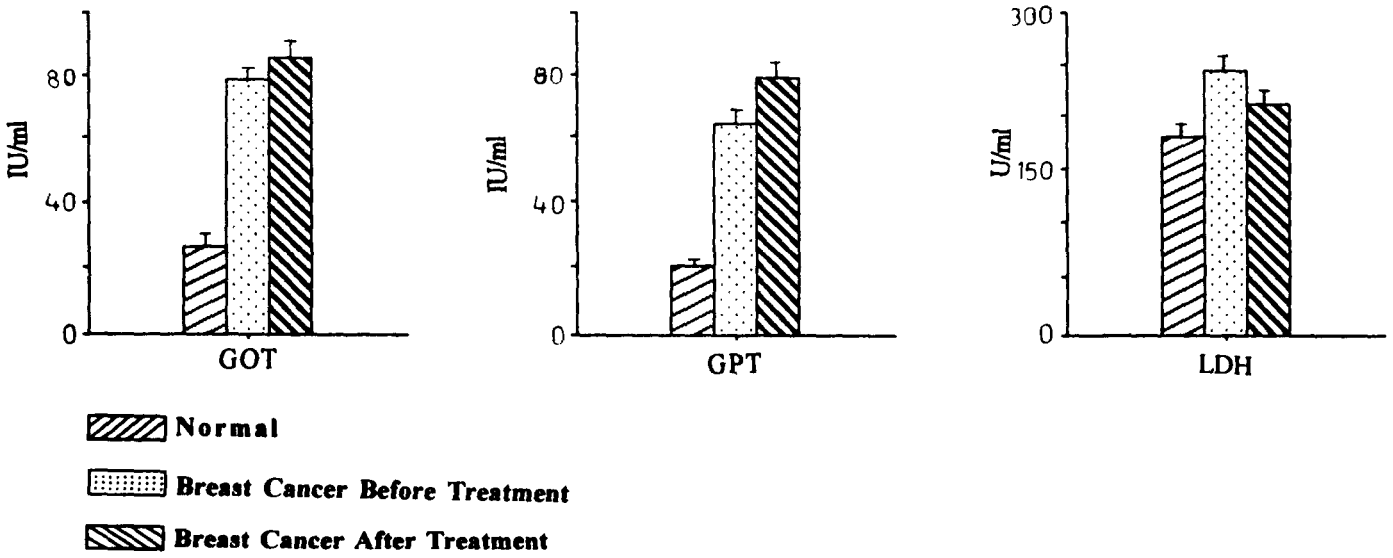


Fig.13

4. **Cancer Liver (15 patients) :**

In the patients of cancer liver the circulating levels of cortisol, GOT, GPT and LDH were raised significantly ($P<0.001$) as compared to controls whereas the activities of RBC AChE and plasma MAO were significantly decreased ($P<0.001$). After one month of therapy (surgery, chemotherapy and radiotherapy) all these parameters remained significantly altered, although the levels reverted towards control values. There was a slight increase in the levels of SGOT and SGPT after treatment (Tables 6 and 7, Figs. 14 and 15).

EXPERIMENTAL

1. **Normal levels of (S-D) female rates**

The circulating activities of AChE, MAO, levels of cortisol, LDH, GOT, GPT and RBC membrane osmotic fragility were estimated in 10 normal female (S-D) rats, given 1ml of sesame oil orally and kept under laboratory conditions (Table-8).

The activities of AChE, SOD and GST and the levels of reduced glutathione (total, free and protein bound) were estimated in various tissues (brain, liver, kidney, heart and spleen) of these control rats and normal values were recorded (Table-8)

In comparison to other tissues, the brain tissues had maximum activity of AChE, while kidney tissues minimum. Maximum activities of SOD and GST were in liver, while minimum activity of GST was recorded in the heart tissues. The brain and spleen tissues showed a low SOD activity than other tissues.

Table - 6

Changes in the circulating levels of AChE, MAO and Cortisol in liver Cancer patients before and after treatment

(Mean ± S.E.M.)

	Normal (35)	Cancer liver patients	
		before treatment (15)	after treatment (12)
RBCs AChE μmole/min/mg proteinx10^{-5}	5.640 ±0.440	3.900[a] ±0.054	4.176[a] ±0.104
Plasma MAO PU/ml	10.934 ±0.214	4.014[a] ±0.292	6.156[a] ±0.144
Plasma Cortisol μg%	16.680 ±1.020	30.926[a] ±1.618	20.026[a] ±0.868

* The numbers in paranthesis indicate the number of patients.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ in comparison to normals.

Changes in the circulating levels of AChE, MAO and Cortisol in Cancer liver patients before and after treatment.

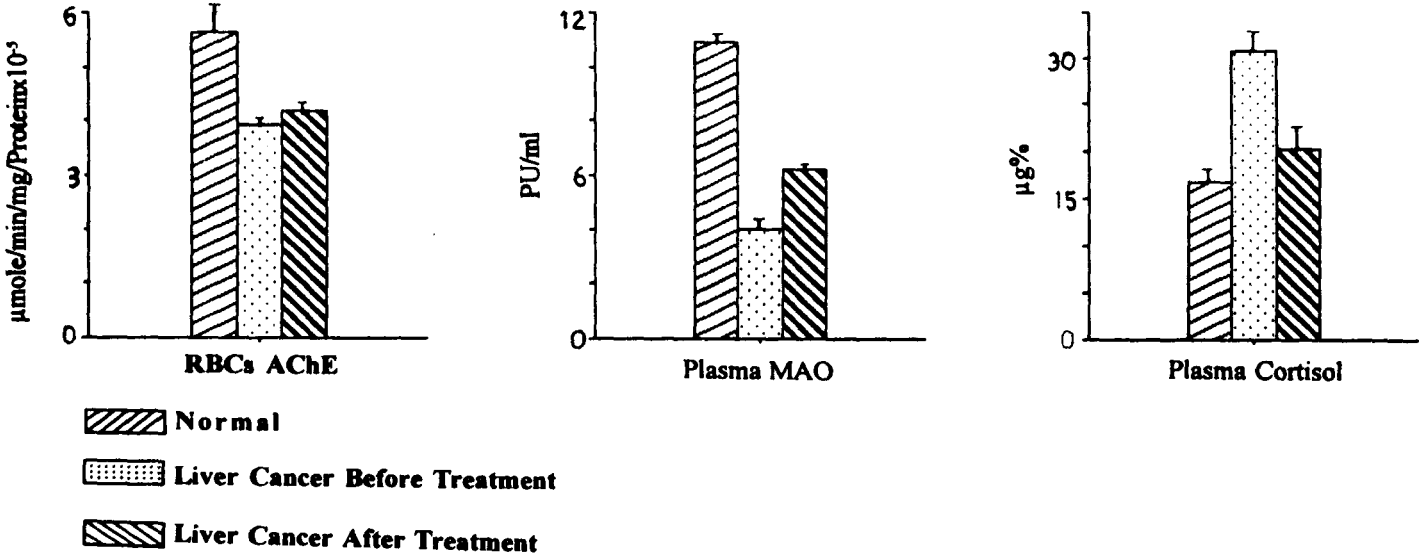


Fig.14

Table - 7

The levels of serum GOT, GPT and LDH in cancer liver patients before and after treatment

(Mean ± S.E.M.)

	Normal (35)	Cancer liver Patients	
		before treatment (15)	after treatment (12)
GOT IU/ml	27.30 ±4.45	182.26[a] ±8.40	189.97[a] ±11.21
GPT IU/ml	24.73 ±2.85	153.19[a] ±7.50	162.06[a] ±6.67
LDH U/ml	187.20 ±11.60	304.18[a] ±20.70	223.70[c] ±12.10

* The numbers in paranthesis indicate the number of patients.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ in comparison to normals.

The levels of GOT, GPT and LDH in Cancer liver patients before and after treatment.

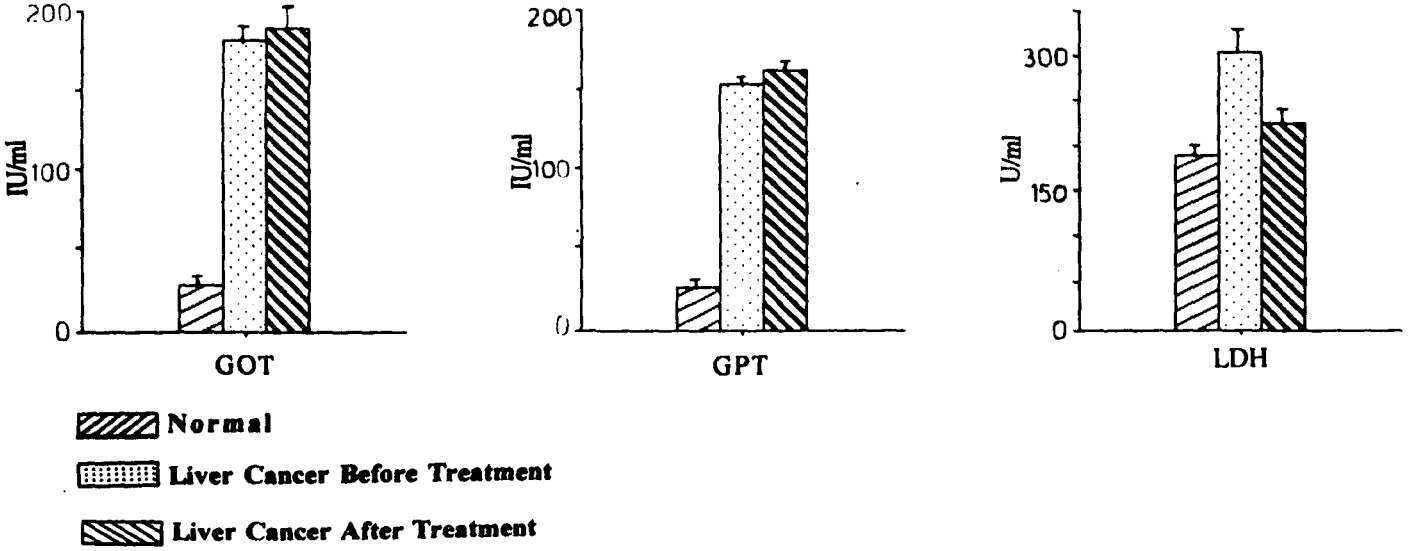


Fig.15

Table - 8

The tissue levels of AChE GST, SOD and total, free and bound GSH in 10 normal rats

(Mean±SEM)

	Brain	Heart	Liver	Kidney	Spleen
AChE (μ moles min/mg prot.x10^{-5})	0.909 ±0.038	0.524 ±0.023	0.188 ±0.012	0.095 ±0.006	0.310 ±0.001
GST (units/mg protein)	161.92 ±2.022	128.08 ±0.962	178.35 ±949	143.46 ±0.695	150.45 ±1.095
SOD (Units/mg tissues)	2.720 ±0.101	9.473 ±0.128	10.813 ±0.057	7.983 ±0.130	2.905 ±0.040
Total GSH (μ mole/gm tissue)	18.280 ±0.660	66.388 ±2.453	87.113 ±2.572	40.973 ±0.920	88.388 ±1.312
Free GHS (μ mole/gm tissue)	5.158 ±0.197	12.840 ±0.283	14.938 ±0.299	11.372 ±0.295	16.393 ±0.110
Protein Bound GSH (μ mole/gm tissue)	13.122 ±0.474	53.499 ±2.216	72.174 ±2.83	29.601 ±0.642	71.995 ±1.095

2(a). Restraint stressed rats

The rats were exposed to restraint stress for different time intervals as 6, 12, 18, 24 and 30 hours to see the maximum effective period. The circulating activities of AChE, MAO were decreased, while the levels of cortisol, GOT, GPT and LDH were increased gradually from 6 to 30 hours of restraint stress. The alterations seen in the activities of RBC AChE and plasma MAO and the levels of cortisol, LDH, GOT and GPT in circulation, were minimum at 6 and 12 hours, while a gradual alterations were recorded at 18 hours and maximum changes were observed at 24 hours. At 30 hours, a slight reversion towards normal was seen in the activities of AChE, MAO and the levels of cortisol and LDH. There was a slight increase in the levels of GOT and GPT at 30 hours of restraint stress treatment (Table-9).

The changes observed in the above mentioned parameters were maximum at 24 hours of stress. Thus, in futher studies, the rats were exposed to 24 hours of restraint stress to see its effect on DMBA induced cancer.

2(b). 24 hours restraint stressed rats

During the 24 hours restraint stress, the activities of RBC AChE, plasma MAO were significantly ($P<0.001$) decreased, while the circulating levels of cortisol, GPT and LDH were significantly elevated ($P<0.001$,0.001 and 0.01 respectively), but there was a less significant increase ($P<0.1$) in serum GOT levels in these rats as compared to controls (Tables 10 and 11, Figs. 16 and 17).

Table - 9

Circulating levels of Cortisol, AChE, MAO, LDH, GOT and GPT in normal and immobilized rats at different time intervals

(Mean ± SEM)

	Nomral (20)	Immobilization (restraint stress)				
		6 hours (20)	12 hours (20)	18 hours (20)	24 hours (20)	30 hours (20)
Plasma Cortisol (µg%)	4.063 ±0.076	4.936[d] ±0.80	5.603[c] ±0.67	6.234[b] ±0.87	6.702[a] ±0.98	6.200[b] ±0.76
RBC AChE (µmole/min/mg protein)x10^{-5}	1.330 ±0.016	1.180[d] ±0.018	1.093[c] ±0.011	1.005[b] ±0.012	0.893[a] ±0.009	0.995[b] ±0.011
Plasma MAO (PU/mL)	2.787 ±0.138	2.438[d] ±0.098	2.246[c] ±0.101	1.987[a] ±0.076	1.932[a] ±0.023	1.980[a] ±0.040
LDH (U/ml)	117.17 ±12.10	138.04[d] ±11.30	144.67[c] ±12.87	158.11[a] ±9.80	160.00[a] ±7.07	157.00[a] ±6.95
SGOT (IU/ml)	13.553 ±1.867	15.430[c] ±1.443	17.186[b] ±1.958	18.395[a] ±1.332	19.480[a] ±2.105	21.340[a] ±1.786
SGPT (IU/ml)	8.863 ±1.195	12.236[c] ±1.670	15.920[b] ±1.363	19.090[a] ±2.080	20.630[a] ±2.175	23.759[a] ±1.976

* The number in paranthesis indicate the number of rats (Samples).

* p a < 0.001, b < 0.01, C < 0.05 and d < 0.1 as compared to normals

Table - 10

Circulating levels of AChE, MAO and Cortisol in control, restraint stressed, DMBA induced cancer only and with pre and post stress treated rats

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
RBC AChE (µmole/min/10/mg proteinx10^{-5})	1.330 ±0.016	0.893[a] ±0.009	0.771 ±0.005	0.727[aa'] ±0.004	0.707[aa'] ±0.002	0.672[aa'] ±0.005
Plasma MAO (PU/ml)	2.787 ±0.138	1.932[a] ±0.023	1.755 ±0.022	1.650[aa'] ±0.019	1.521[aa'] ±0.016	1.388[aa'] ±0.021
Plasma Cortisol (µg%)	4.063 ±0.076	6.702[a] ±0.098	7.261 ±0.120	7.533[aa'] ±0.105	8.194[aa'] ±0.127	8.977[aa'] ±0.148

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ as compared to stress.

Circulating levels of AChE, MAO and Cortisol in control, restraint stressed, DMBA induced Cancer (sacrificed after 130 days) in rats.

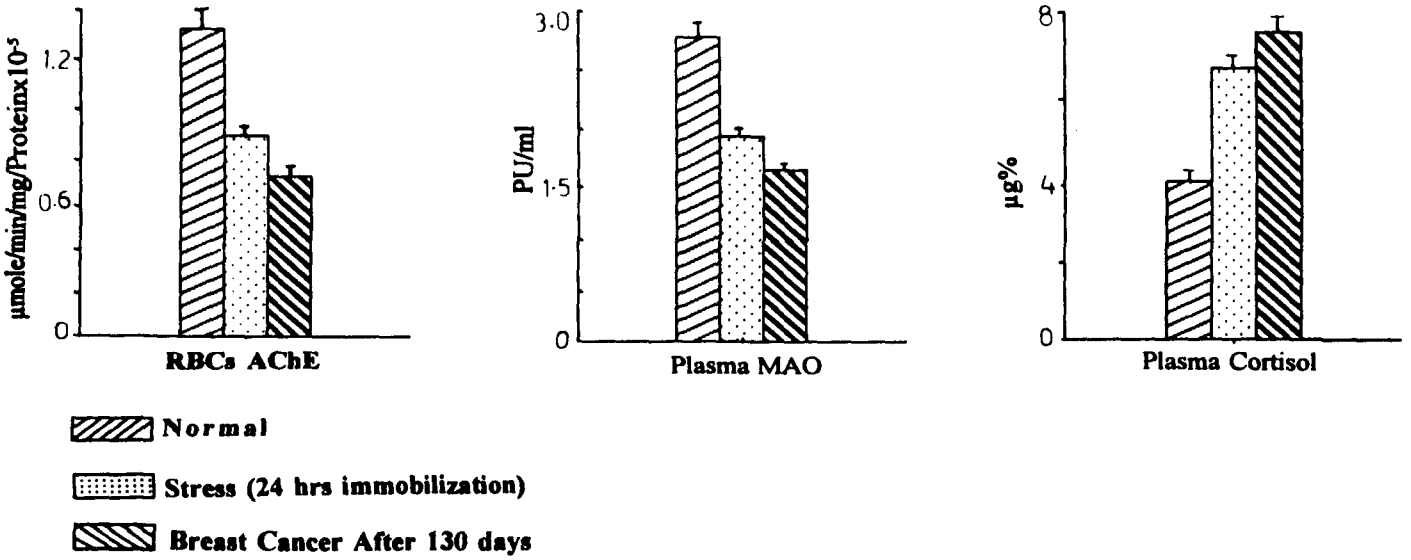


Fig. 16

Table - 11

Serum levels of LDH, GOT and GPT in normal, restraint stressed, DMBA induced Cancer only and with pre and post stress treated rats.

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
LDH (U/ml)	117.17 ±12.00	160.80^{b} ±7.071	179.19 ±8.904	187.00$^{aa'}$ ±12.564	209.00$^{aa'}$ ±12.219	215.50$^{aa'}$ ±3.135
SGOT (IU/ml)	13.553 ±1.867	19.480^{d} ±2.105	41.531 ±1.650	49.260$^{aa'}$ ±1.581	53.36$^{aa'}$ ±1.731	58.060$^{aa'}$ ±1.332
SGPT (IU/ml)	8.863 ±1.195	12.630^{b} ±2.175	33.960 ±1.660	40.890$^{ab'}$ 1.453	43.500$^{aa'}$ ±1.345	49.730$^{aa'}$ ±1.097

* The numbers in paranthesis indicate the number of rats (Samples).
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ in comparison to stress.

Serum levels of LDH, GOT and GPT in normal, restraint stressed and DMBA induced Cancerous (sacrificed after 130 days) in rats.

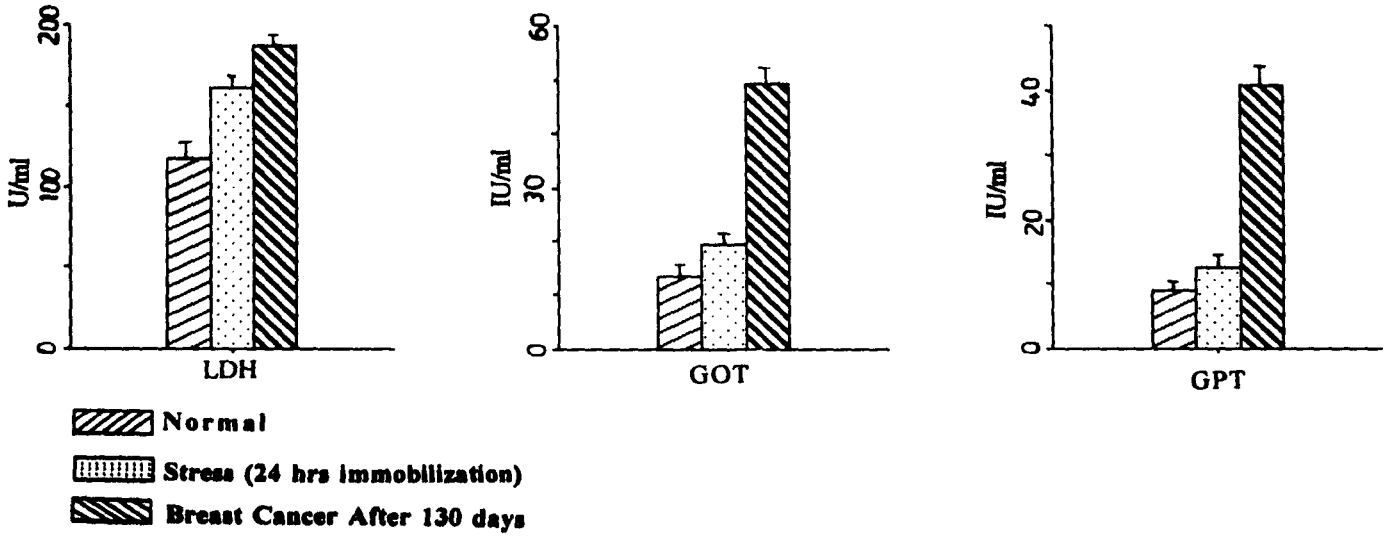


Fig.17

The activities of AChE, GST and SOD, the levels of total, free and protein bound GSH were significantly decreased ($P<0.001$) in the tissues (heart, liver, kidney, spleen and brain) of restraint stressed rats. as compared to the values obtained from the corresponding tissues of their respective normal rats (Tables 12-21, Figs. 18-27).

3. DMBA induced mammary cancer in female rats

An oral infusion of DMBA solution. (30 mg/kg body weight, single dose treatment) was given to female (S-D) rats. After 130 days, heparinised blood and various tissues (heart, brain, liver, kidney and spleen) were collected immediately after sacrificing the rats by giving pentobarbital (50 mg/kg body weight intraperitoneal) as described earlier. The blood was subjected for the estimation of AChE, MAO, cortisol and erythrocyte membrane osmotic fragility, while tissues for the activities of AChE, GST and SOD and the levels of total, free and protein bound GSH.

In the DMBA treated (cancerous)rats, the circulating activities of AChE and MAO were significantly decreased ($P<0.001$), while the levels of cortisol,GOT,GPT and LDH were significantly elevated ($P<0.001$) as compared to either the control or restraint stressed rats (Tables 10 and 11, Figs. 16 and 17).

As compared to the respective normal or restraint stress tissue values, the activites of AChE, GST and SOD, the levels of total, free and protein bound GSH were significantly decreased ($P<0.001$) in various tissues (heart, liver, kidney, brian and spleen) of DMBA treated rats (Tables 12-21, Figs. 18-27).

Table - 12

The heart tissue levels of AChE, SOD and GST in normal, restraint stressed, DMBA induced Cancer only and with pre and post stress treated rats.

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
Tissue AchE (μmole/min/mg protein x 10^{-5})	0.524 ±0.023	0.331[a] ±0.005	0.312 ±0.005	0.300[aa'] ±0.004	0.259[aa'] ±0.004	0.206[aa'] ±0.006
Tissue SOD (Units/mg)	9.473 ±0.129	7.869[a] ±0.135	6.459 ±0.086	6.240[aa'] ±0.077	5.487[aa'] ±0.049	4.032[aa'] ±0.082
Tissue GST (Units/mgprotein)	128.08 ±0.962	93.35[a] ±0.660	83.93 ±0.420	79.46[aa'] ±0.316	74.74[aa'] ±0.401	71.55[aa'] ±0.393

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ in comparison to stress.

The heart tissue levels of AChE, SOD and GST in normal, restraint stressed and DMBA induced Cancerous (sacrificed after 130 days) in rats.

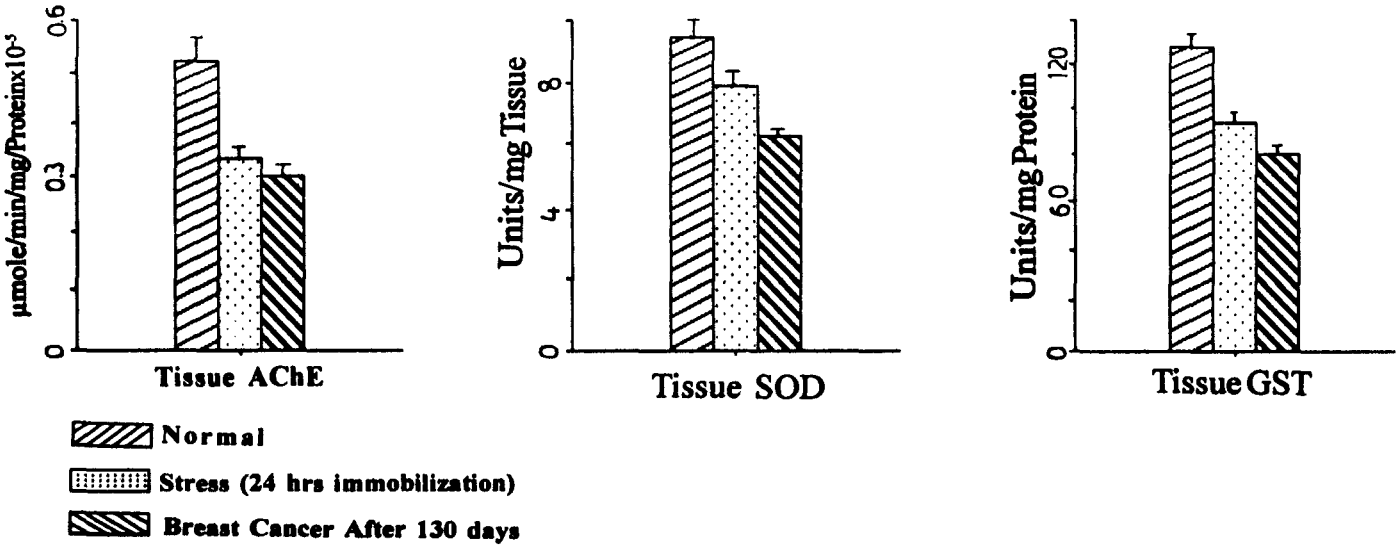


Fig.18

Table - 13

The levels of total, free and protein bound GSH in normal, restraint stressed, and DMBA induced cancer only and with pre and post stress treatment in heart tissues of rats.

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
Total GSH (μ mole/gm of tissues)	6.638 ±0.245	4.962[a] ±0.066	4.305 ±0.086	4.004[aa'] ±0.102	3.424[aa'] ±0.245	2.931[aa'] ±0.040
Free GSH (μ Mole/gm of Tissue)	1.289 ±0.028	0.990[a] ±0.020	0.893 ±0.016	0.857[aa'] ±0.014	0.765[aa'] ±0.007	0.728[aa'] ±0.005
Prot.bound GSH (μmole/ gm of tissue)	5.350 ±0.222	3.992[a] ±0.090	3.319 ±0.109	3.148[ab'] ±0.190	2.654[aa'] ±0.099	2.193[aa'] ±0.035

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ as compared to stress.

The levels of total, free and protein bound GSH in heart tissues of normal, restraint stressed and DMBA induced cancer (sacrificed after 130 days) in rats.

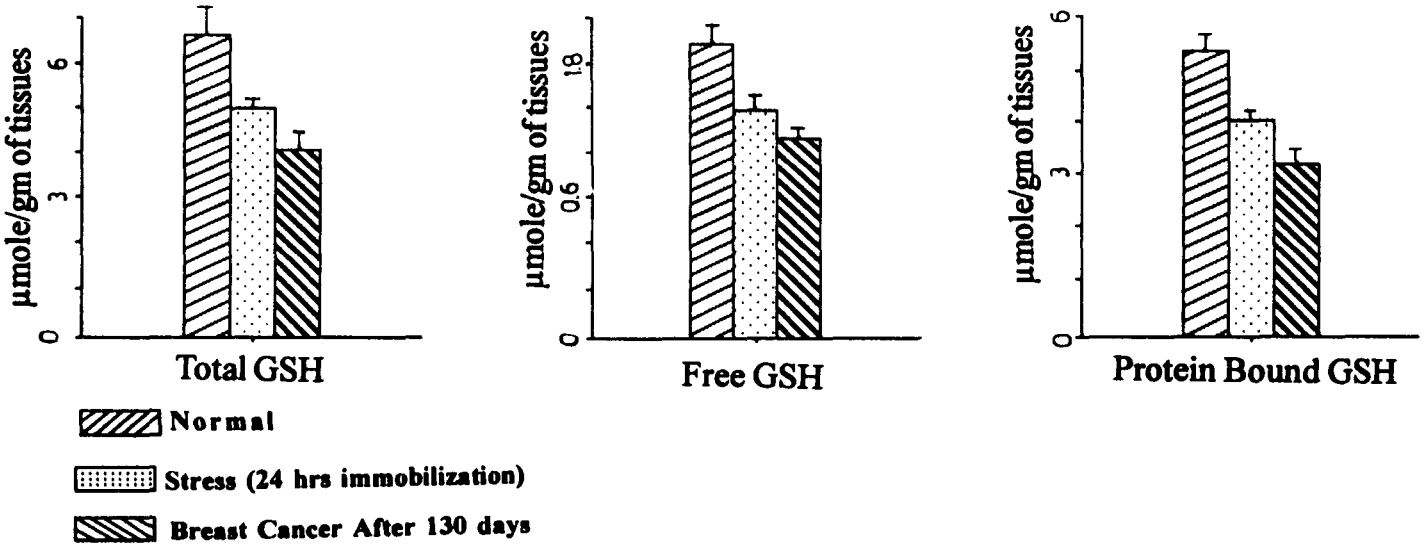


Fig. 19

Table - 14

The levels AChE, GST and SOD in liver tissue of control, restraint stressed, DMBA induced cancer and DMBA infusion with pre and post stress treated rats.

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
Tissue AChE (μ mole/min/mg protein x 10^{-5})	0.188 ±0.012	0.126[a] ±0.002	0.121 ±0.001	0.118[ac'] ±0.001	0.106[aa'] ±0.001	0.090[aa'] ±0.002
Tissue SOD (Units/mg Tissue)	10.813 ±0.058	10.004[a] ±0.075	9.134 ±0.051	8.685[aa'] ±0.024	8.576[aa'] ±0.034	8.373[aa'] ±0.032
Tissue GST (Units/mg protein)	178.35 ±0.949	136.87[a] ±0.655	117.57 ±0.568	109.30[aa'] ±0.732	103.94[aa'] ±0.615	99.50[aa'] ±0.593

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ in comparison to stress.

The levels of AChE, GST and SOD in liver tissue of control, restraint stressed and DMBA induced cancer (sacrificed after 130 days) in rats.

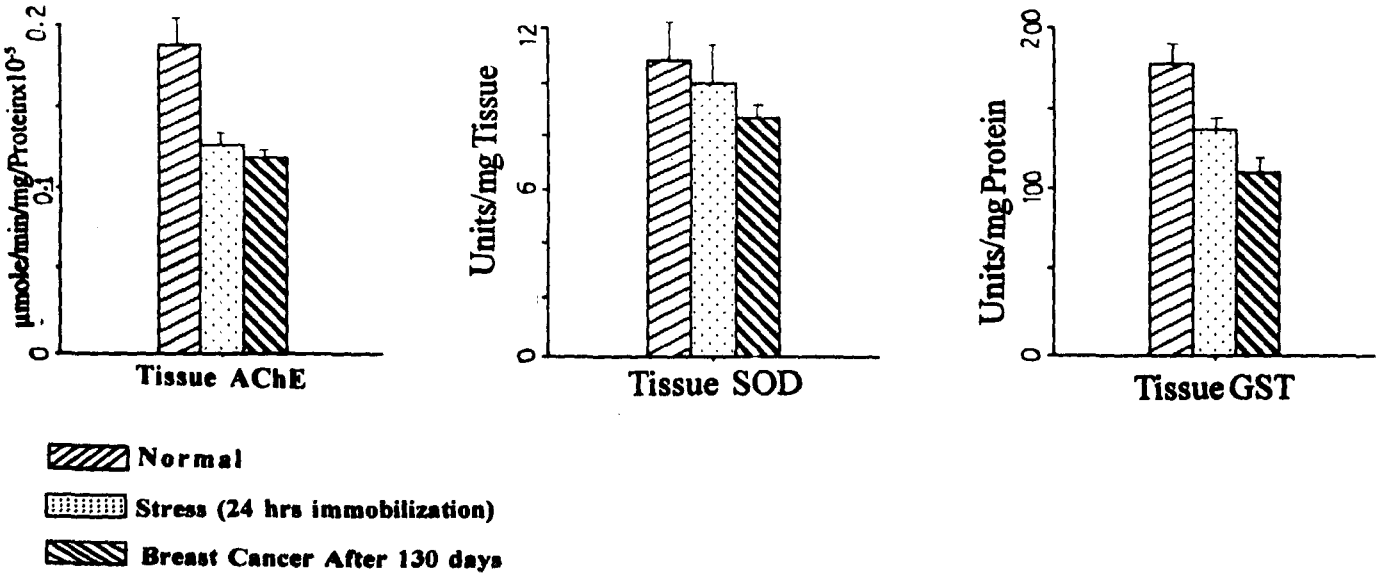


Fig. 20

Table - 15

The rat liver tissue levels of total, free and protein bound GSH in control, restraint stressed, DMBA induced cancer and DMBA induced cancer with pre and post stress treated rats

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
Total GSH (μ mole/gm of tissues)	8.711 ±0.257	6.721^{a} ±0.138	6.287 ±0.199	6.047$^{ac'}$ ±0.245	5.629$^{aa'}$ ±0.069	5.235$^{aa'}$ ±0.034
Free GSH (μ Mole/gm of Tissue)	1.493 ±0.030	1.198^{a} ±0.039	1.081 ±0.020	1.042$^{ad'}$ ±0.034	0.939$^{aa'}$ ±0.004	0.879$^{aa'}$ ±0.010
Prot.bound GSH (μ mole/gm of tissue)	7.217 ±0.228	5.510^{c} ±0.010	5.180 ±0.020	5.005$^{ac'}$ ±0.039	4.690$^{aa'}$ ±0.064	4.355$^{aa'}$ ±0.024

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ in comparison to stress.

The rat liver tissue levels of total, free and protein bound GSH in control, restraint stressed and DMBA induced cancer (sacrificed after 130 days) in rats.

treated rats.

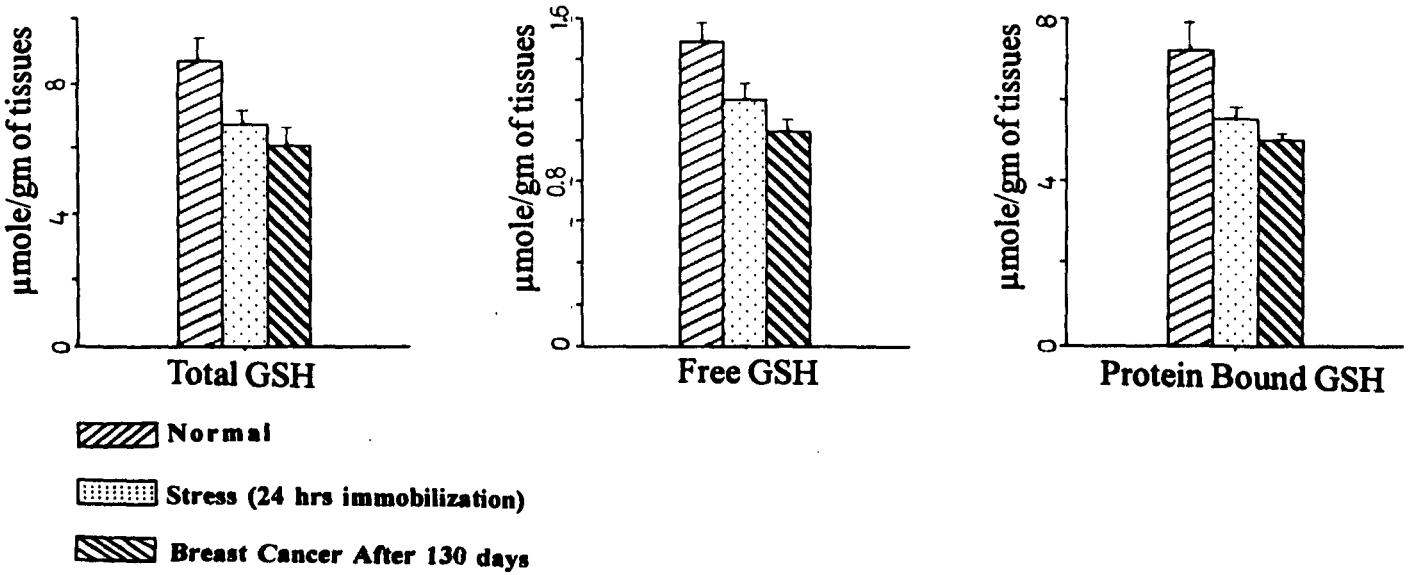


Fig. 21

Table - 16

The levels of AChE, SOD and GST in the kidney tissues of normal, restraint stressed, DMBA induced cancerous alone and with pre and post stress treated rats.

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
Tissue AChE (μmole/min/mg prot. x 10^{-5})	0.095 ±0.006	0.067^{b} ±0.001	0.061 ±0.002	$0.057^{aa'}$ ±0.001	$0.046^{aa'}$ ±0.002	$0.039^{aa'}$ ±0.001
Tissue SOD (Units/mg Tissue)	7.983 ±0.014	6.190^{a} ±0.034	5.824 ±0.060	$5.747^{aa'}$ ±0.058	$4.163^{aa'}$ ±0.099	$3.464^{aa'}$ ±0.080
Tissue GST (Units/mgprotein)	143.46 ±0.695	123.65^{a} ±0.648	96.18 ±0.871	$89.90^{aa'}$ ±0.418	$85.89^{aa'}$ ±0.386	$79.95^{aa'}$ ±0.323

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ in comparison to stress.

The levels of AChE, SOD and GST in the kidney tissues of normal, restraint stressed and DMBA induced cancer (sacrificed after 130 days) in rats.

Fig. 22

Table - 17

The kidney tissue levels of total, free and protein bound GSH in normal, restraint stressed, DMBA induced cancer only and with pre and post stress treated rats.

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
Total GSH (μ mole/gm of tissues)	4.097 ±0.092	3.348[a] ±0.082	2.936 ±0.073	2.855[aa'] ±0.043	2.641[aa'] ±0.025	2.436[aa'] ±0.022
Free GSH (μ Mole/gm of Tissue)	1.137 ±0.030	0.957[a] ±0.020	0.890 ±0.010	0.876[ab'] ±0.006	0.817[aa'] ±0.006	0.742[aa'] ±0.021
Protein bound GSH (μmole/gm of tissue)	2.960 ±0.164	2.395[a] ±0.166	2.117 ±0.110	1.981[aa'] ±0.139	1.825[aa'] ±0.019	1.694[aa'] ±0.007

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ in comparison to stress.

The kidney tissue levels of total, free and protein bound GSH in normal, restraint stressed and DMBA induced cancer (*sacrificed after 130* days) in rats.

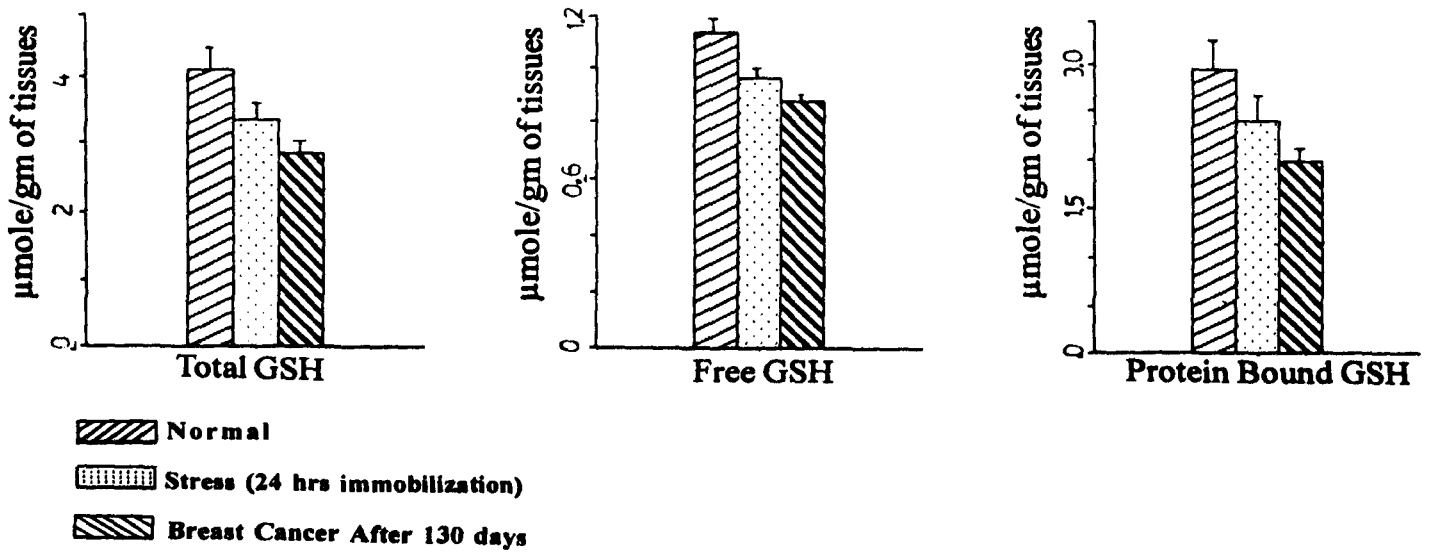


Fig. 23

Table - 18

The brain tissue levels of AChE, SOD and GST in normal, restraint stressed, DMBA induced cancer only and with pre and post stress treated rats

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
Tissue AchE (μ mole/min/mg protein x 10^{-5})	0.909 ±0.038	0.608[a] ±0.008	0.532 ±0.006	0.500[aa'] ±0.006	0.448[aa'] ±0.005	0.402[aa'] ±0.009
Tissue SOD (Units/mg Tissue)	2.720 ±0.001	1.922[a] ±0.040	1.787 ±0.030	1.722[ab'] ±0.026	1.576[aa'] ±0.020	1.390[aa'] ±0.043
Tissue GST (Units/mgprotein)	161.92 ±0.022	128.08[a] ±1.758	105.45 ±1.980	99.95[aa'] ±1.456	94.45[aa'] ±0.963	90.16[aa'] ±0.877

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ in comparison to stress.

The brain tissue levels of AChE, SOD and GST in normal, restraint stressed and DMBA induced cancer (sacrificed after 130 days) in rats.

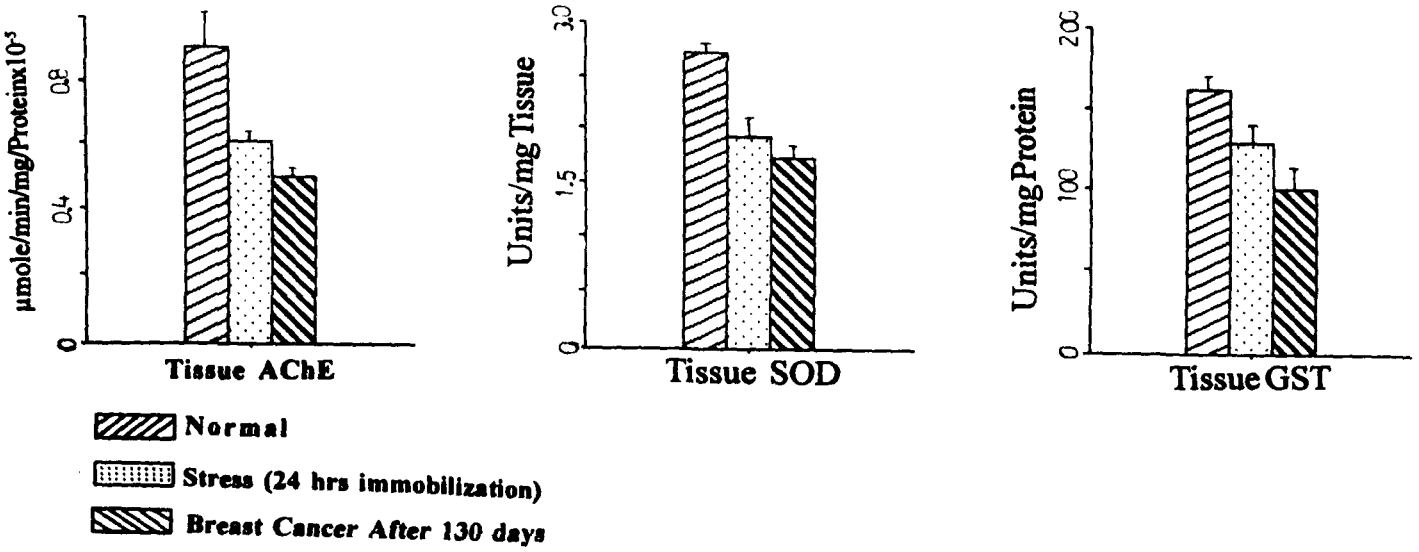


Fig. 24

Table - 19

The brain tissue levels of total, free and protein bound GSH in normal, restraint stressed, DMBA iduced cancer only and with pre and post stress treatment

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
Total GSH (μ mole/gm of tissues)	1.828 ±0.066	1.347[a] ±0.036	1.169 ±0.025	1.097[aa'] ±0.024	0.926[aa'] ±0.015	0.869[aa'] ±0.022
Free GSH (μ Mole/gm of tissue)	0.516 ±0.020	0.390[b] ±0.010	0.367 ±0.009	0.353[ab'] ±0.002	0.334[aa'] ±0.005	0.308[aa'] ±0.005
Protein bound GSH (μ mole/gm of tissue)	1.312 ±0.047	0.957[a] ±0.025	0.811 ±0.022	0.747[aa'] ±0.019	0.625[aa'] ±0.011	0.616[aa'] ±0.005

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ as compared to stress.

The brain tissue levels of total, free and protein bound GSH in normal, restraint stressed and DMBA *induced cancer (sacrificed after 130 days) in rats.*

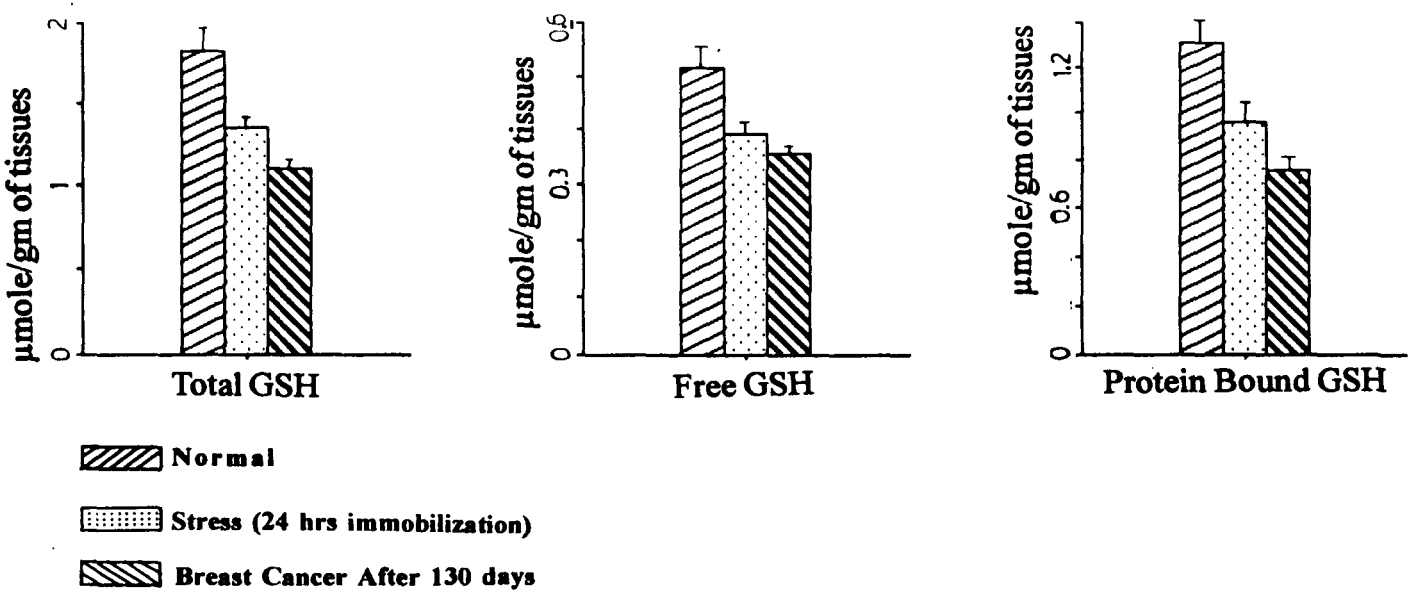


Fig. 25

Table - 20

The spleen tissue levels of AChE, SOD and GST in normal, restraint stressed, DMBA induced cancer only and with pre and post stress treatment

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
Tissue AChE (μ mole/min/mg protein x 10^{-5})	0.310 ±0.001	0.221[a] ±0.005	0.197 ±0.003	0.191[aa'] ±0.002	0.178[aa'] ±0.002	0.151[aa'] ±0.004
Tissue SOD (Units/mg tissue)	2.905 ±0.040	2.526[a] ±0.028	2.382 ±0.019	2.322[aa'] ±0.025	2.084[aa'] ±0.026	2.027[aa'] ±0.041
Tissue GST (Units/mgprotein)	150.45 ±0.095	118.04[a] ±0.805	96.26 ±0.904	91.50[aa'] ±0.736	89.16[aa'] ±0.933	83.93[aa'] ±0.811

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ in comparison to stress.
* a'' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d'' $p<0.1$ in comparison to110 days DMBA induced cancer.

The spleen tissue levels of AChE, SOD and GST in normal, restraint stressed and DMBA induced cancerous (sacrificed after 130 days) in rats.

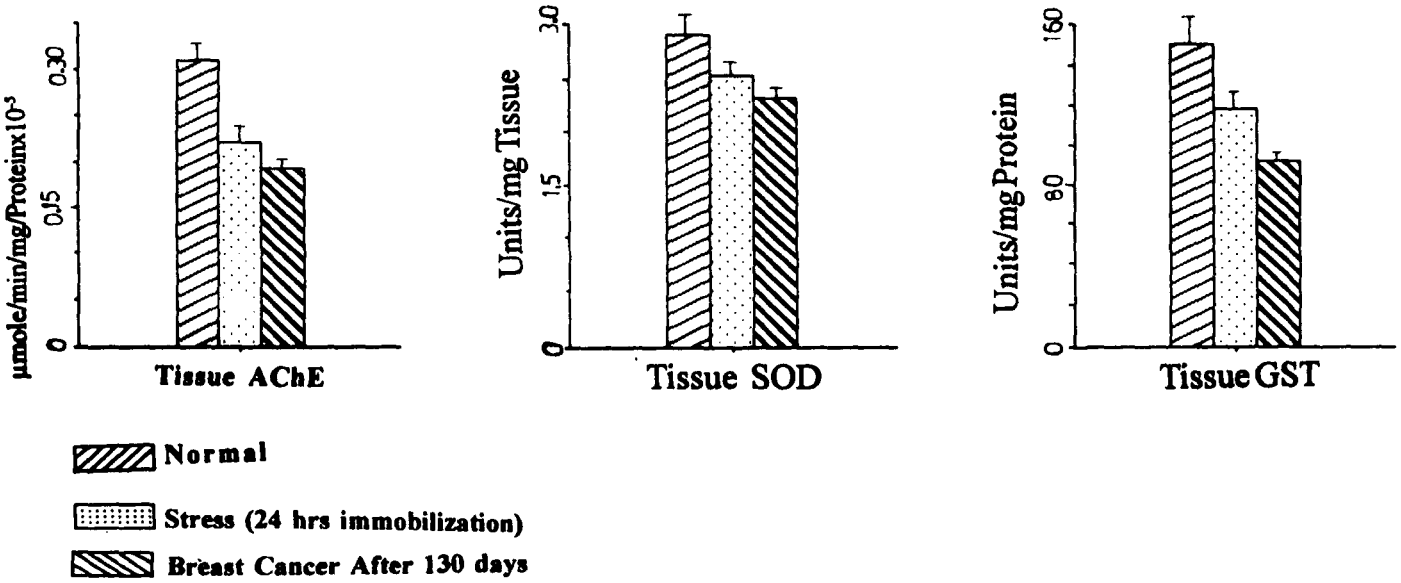


Fig. 26

Table - 21

The levels of total, free and protein bound GSH in spleen tissue of normal, restraint stressed, DMBA iduced cancer only and with pre and post stress treatment

(Mean ± S.E.M.)

	Normal (10)	Stress (10)	DMBA Induced Cancer		DMBA infusion with	
			after 110 days (10)	after 130 days (10)	Pre-stress Treatment (10)	Post-stress Treatment (10)
Total GSH (μ mole/gm of tissues)	8.839 ±1.031	7.376^{a} ±1.064	6.696 ±0.908	6.529$^{ab'}$ ±1.023	5.966$^{aa'}$ ±0.082	5.496$^{aa'}$ ±0.080
Free GSH (μ Mole/gm of tissue)	1.639 ±0.011	1.190^{a} ±0.040	1.005 ±0.030	0.988$^{ab'}$ ±0.013	0.904$^{aa'}$ ±0.007	0.814$^{aa'}$ ±0.009
Prot.bound GSH (μ mole/gm of tissue)	7.199 ±0.110	6.186^{c} ±0.131	5.705 ±0.109	5.537$^{ab'}$ ±0.110	5.061$^{aa'}$ ±0.075	4.682$^{aa'}$ ±0.072

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ in comparison to stress.

The levels of total, free and protein bound GSH in spleen tissues of normal, restraint stressed and DMBA induced cancer (sacrificed after 130 days) in rats.

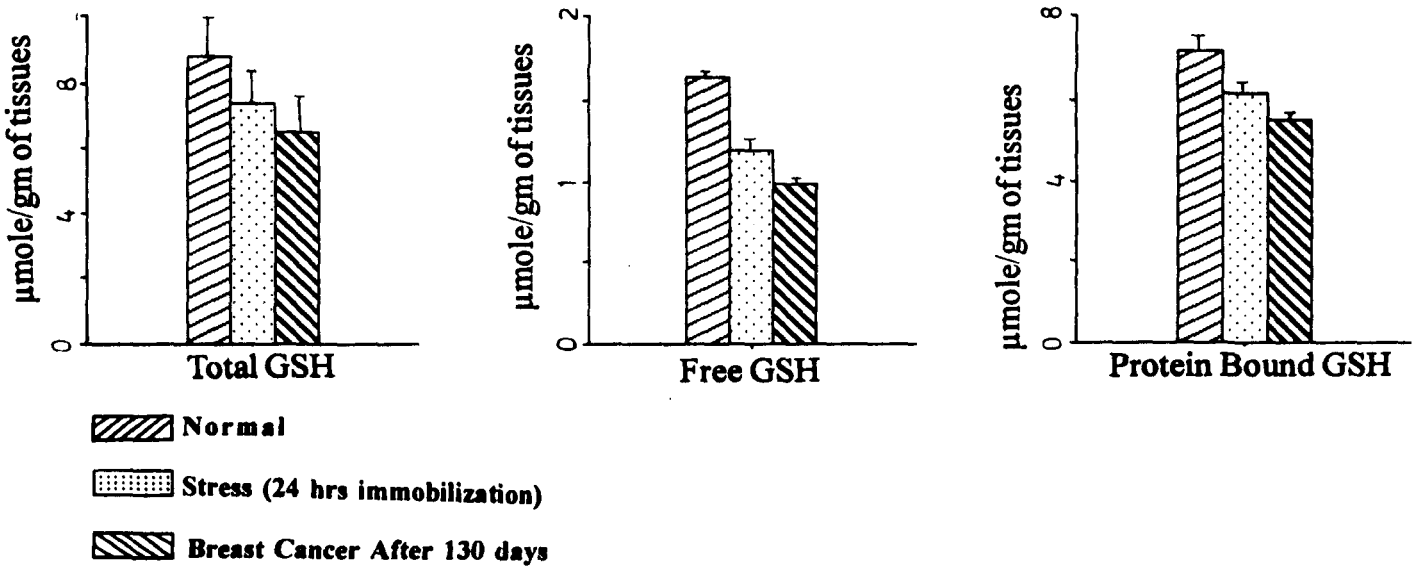


Fig. 27

4. Effect of stress on DMBA induced cancer in rats

4(a). The effect of pre-stress treatment on DMBA induced cancer

In this group, each rat was immobilized indvidually for 24 hours prior to the single dose administration of DMBA (30 mg/Kg body weight, by gavage) as described earlier.

The circulating activities of AChE and MAO were significantly decreased ($P< 0.001$), while the levels of cortisol, GOT, GPT and LDH were significantly ($P< 0.001$) elevated in DMBA infused pre-stress treated rats as compared to the values obtained from their respective restraint stressed or DMBA only infused rats (Tables 10 and 11, Figs. 28 and 29).

The activities of AChE, GST and SOD, the levels of total, free and protein bound GSH were significantly ($P<0.001$) decreased in heart, kidney, liver, brain and spleen tissues of DMBA infused pre-stress treated rats when compared to their respective levels from the heart, kidney, liver, brain and spleen tissues of restraint stressed or only DMBA infused rats. (Tables 12-21, Figs. 30-39).

4(b): Effect of post-stress treatment on DMBA induced cancer

In this group, each rat was immobilized indvidually for 24 hours after the single-dose administration of DMBA (30 mg/Kg body weight, by gavage), as described earlier.

The tissues (heart, brain, liver, kidney and spleen), and blood (erythrocytes, plasma and serum) were immediately collected after sacrificing the rats and they were subjected for the assay of the biochemical parameters as described earlier.

Circulating levels of AChE, MAO and Cortisol in control, restraint stressed, DMBA induced Cancerous (sacrified after 110 days) and DMBA infused rats with pre and post stress treatment.

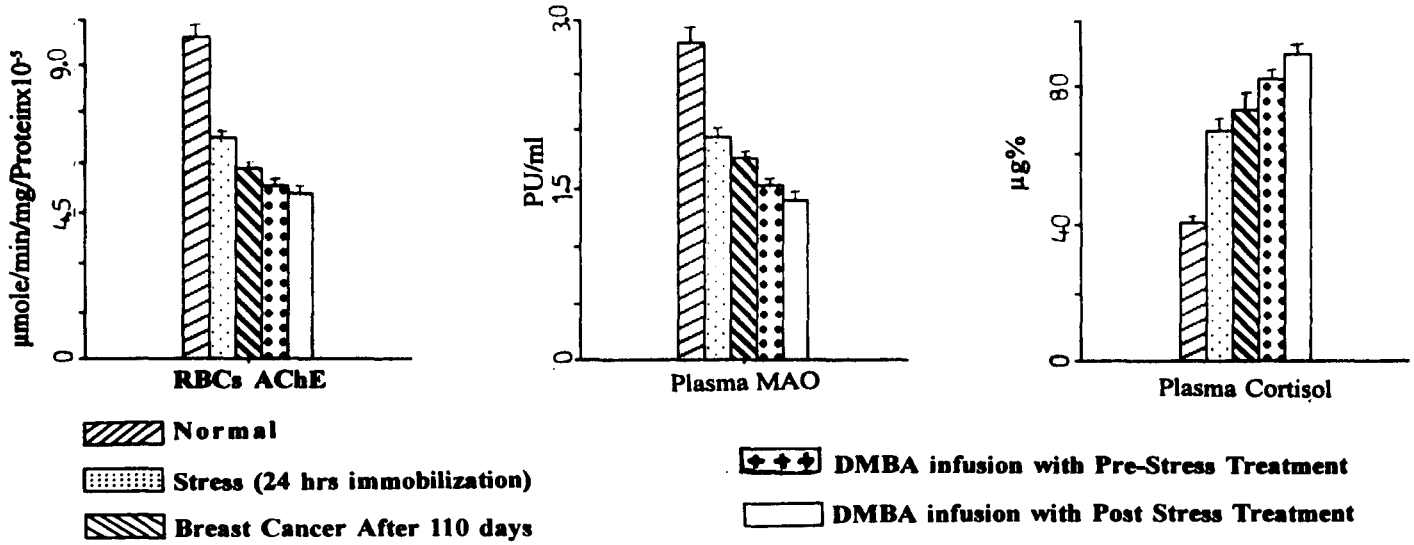


Fig. 28

Serum levels of LDH, GOT and GPT in normal, restraint stressed, DMBA induced Cancer (sacrificed after 110 days) and DMBA infused rats with pre and post stress treatment.

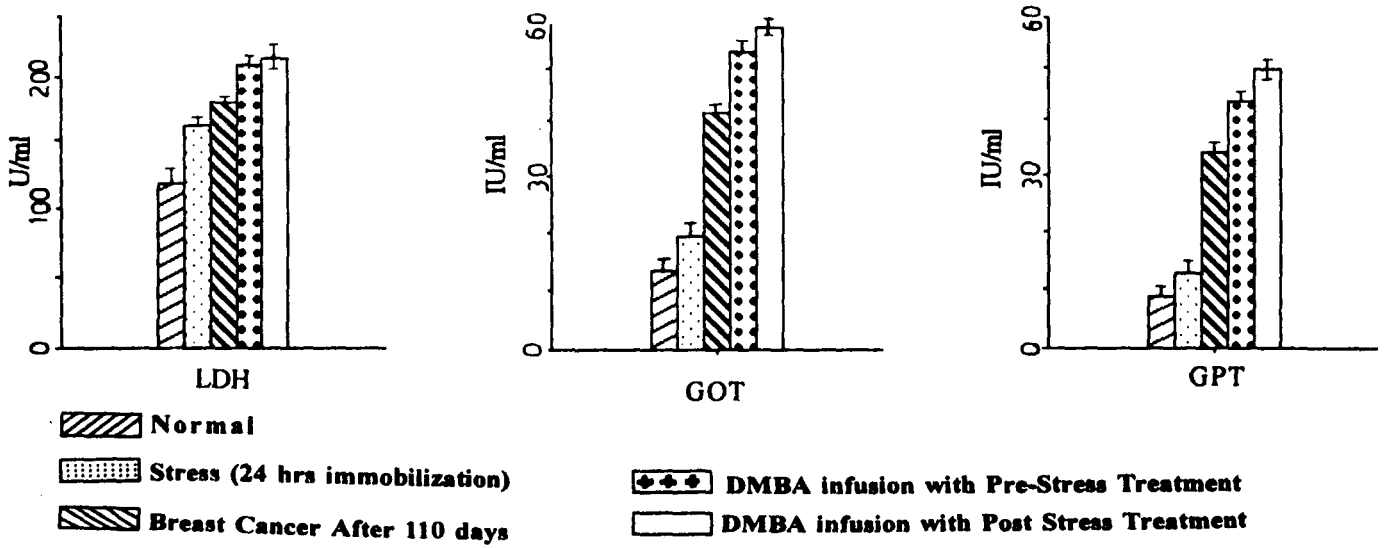


Fig. 29

The heart tissue levels of AChE, SOD and GST in normal, restraint stressed, DMBA induced cancer (sacrificed after 110 days) and DMBA infusion with pre and post stress treated rats.

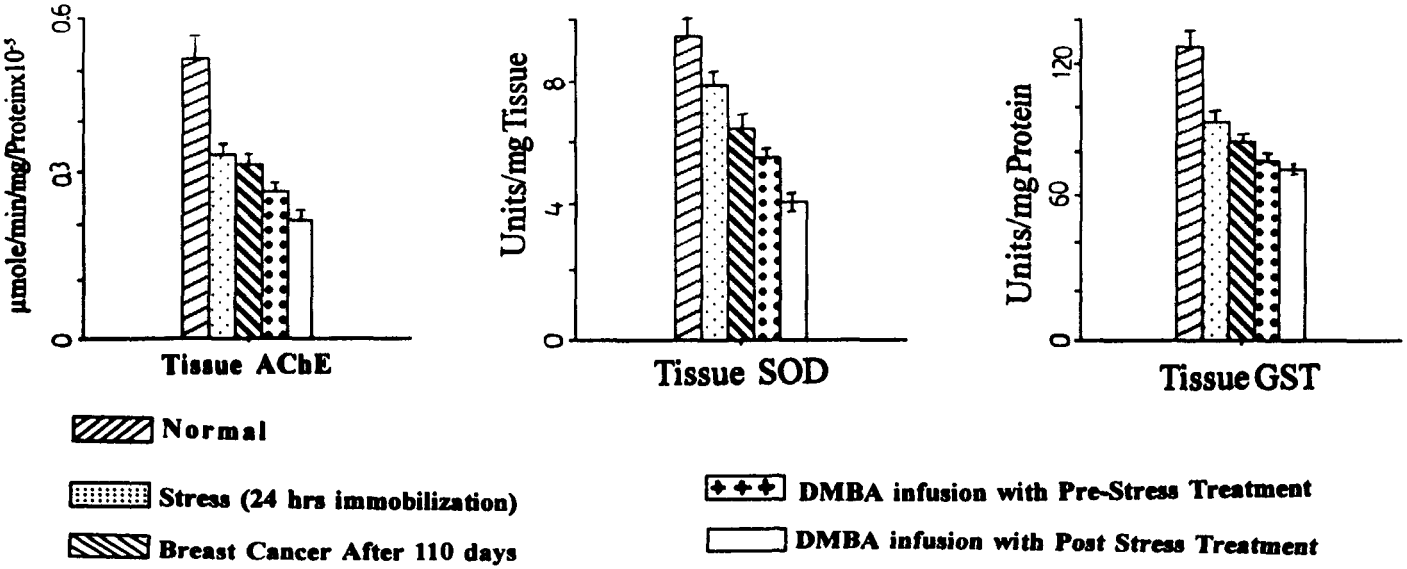


Fig. 30

The levels of total, free and protein bound GSH in heart tissues of normal, restraint stressed, DMBA induced cancer (sacrificed after 110 days) and DMBA infusion with pre and post stress treated rats.

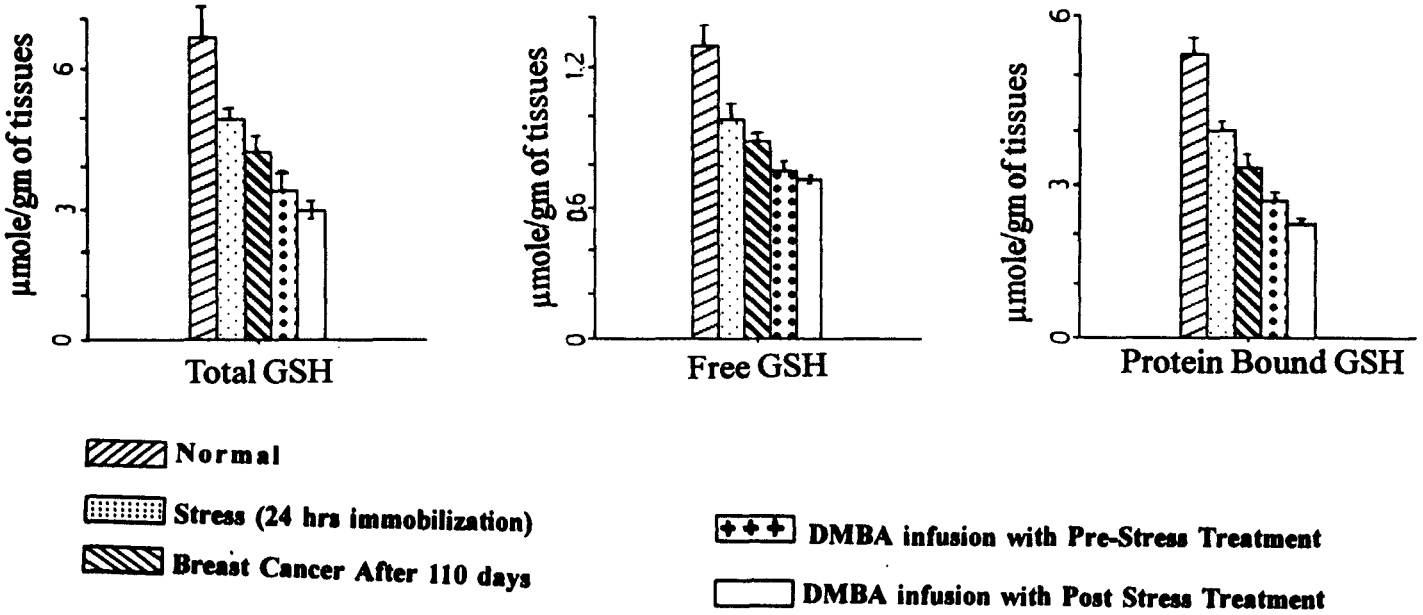


Fig. 31

The levels of AChE, GST and SOD in liver of control, restraint stressed, DMBA induced cancer (sacrificed after 110 days and DMBA infusion with pre and post stress treated rats.

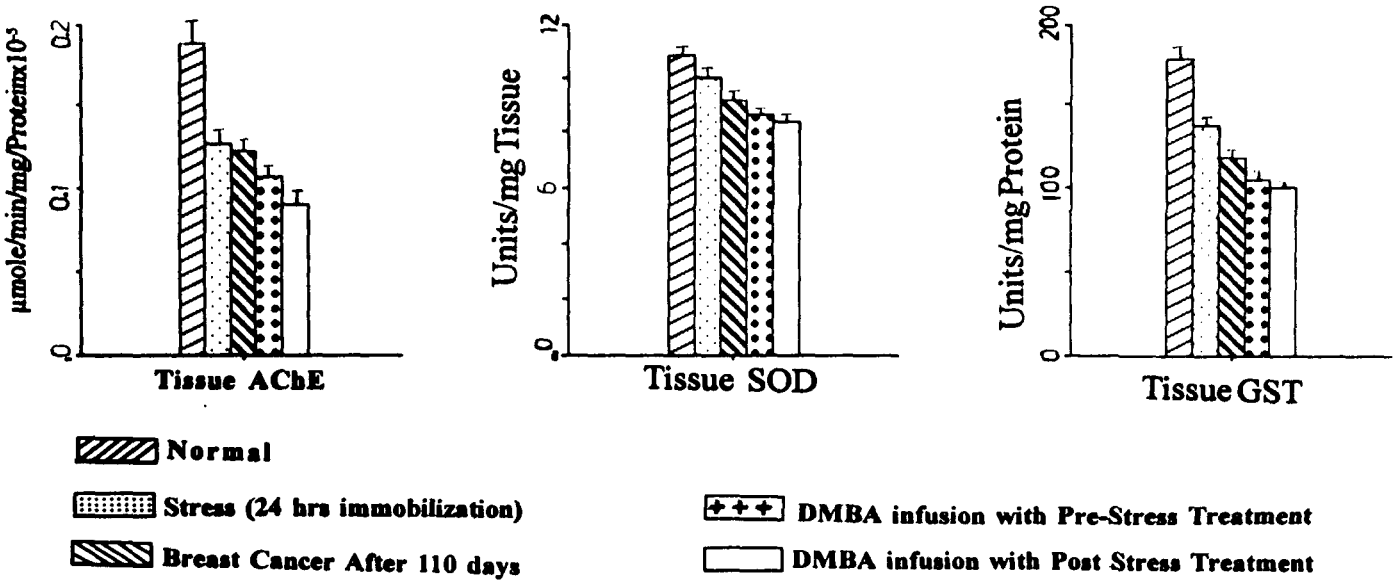


Fig. 32

The rat liver tissue levels of total, free and protein bound GSH in control, restraint stressed, DMBA induced cancer (sacrificed after 110 days) and DMBA infusion with pre and post stress

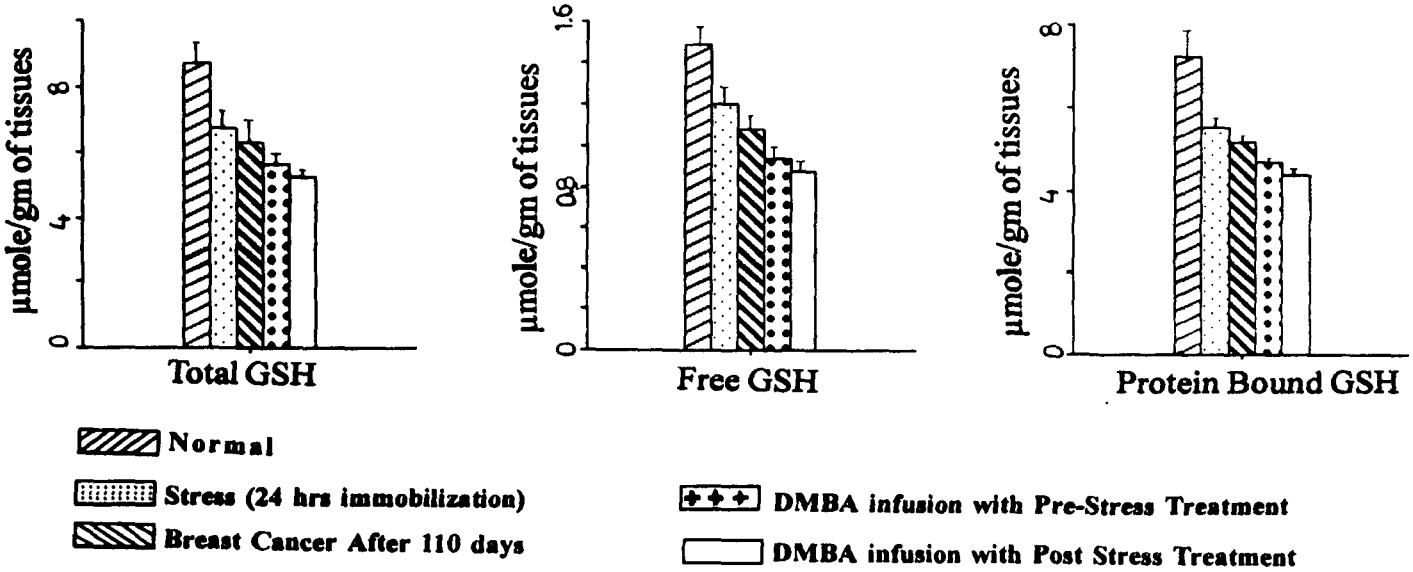


Fig. 33

The levels of AChE, SOD and GST in the kidney tissues of normal, restraint stressed, DMBA induced cancer (sacrificed after 110 days) and DMBA infusion with pre and post stress treated rats.

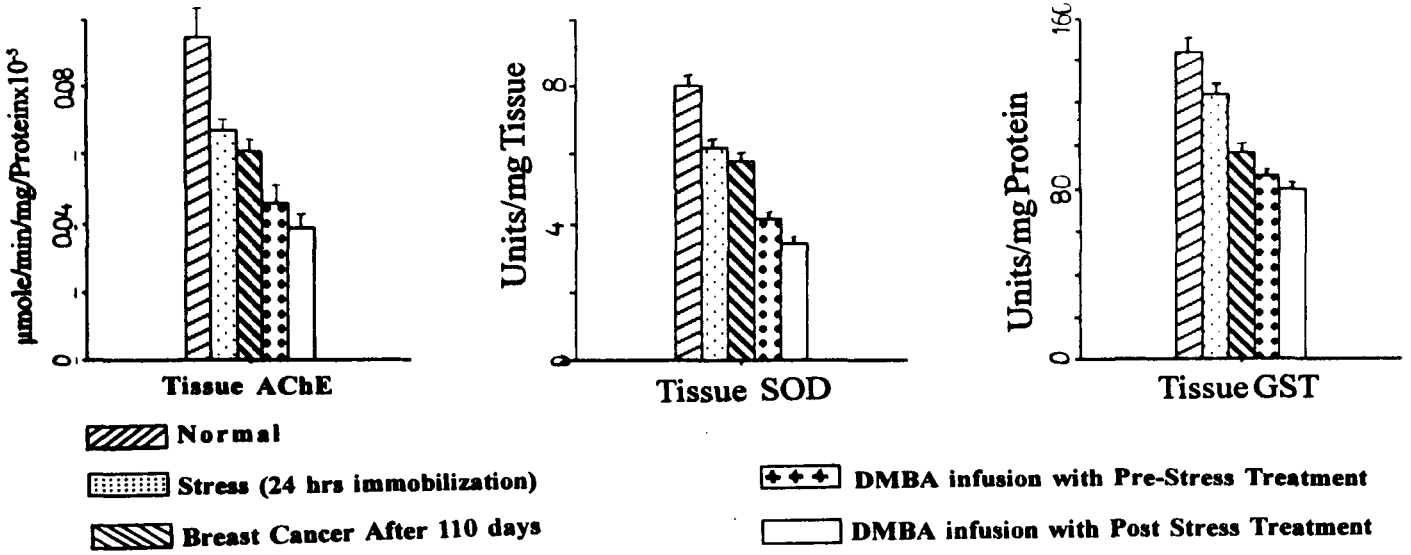


Fig. 34

The kidney tissue levels of total, free and protein bound GSH in normal, restraint stressed, DMBA induced cancer (sacrificed after 110 days) and DMBA infusion with pre and post and stress treated rats.

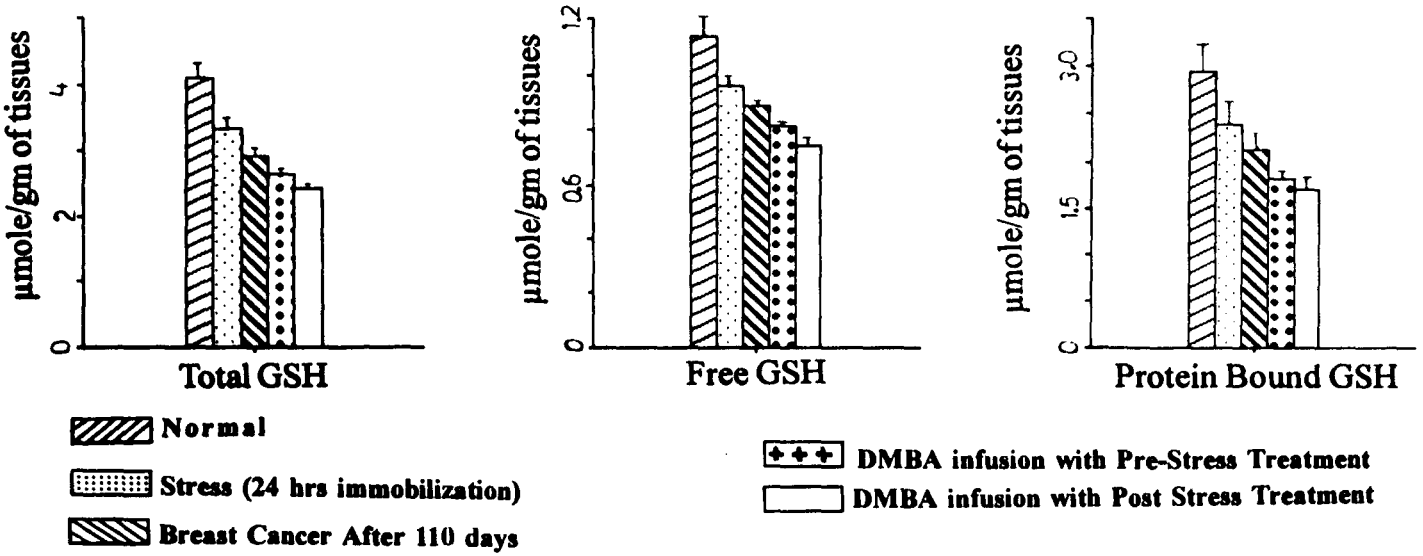


Fig. 35

The brain tissue levels of AChE, SOD and GST in normal, restraint stressed, DMBA induced cancerous (sacrificed after 110 days) and DMBA infusion with pre and post stress treated rats.

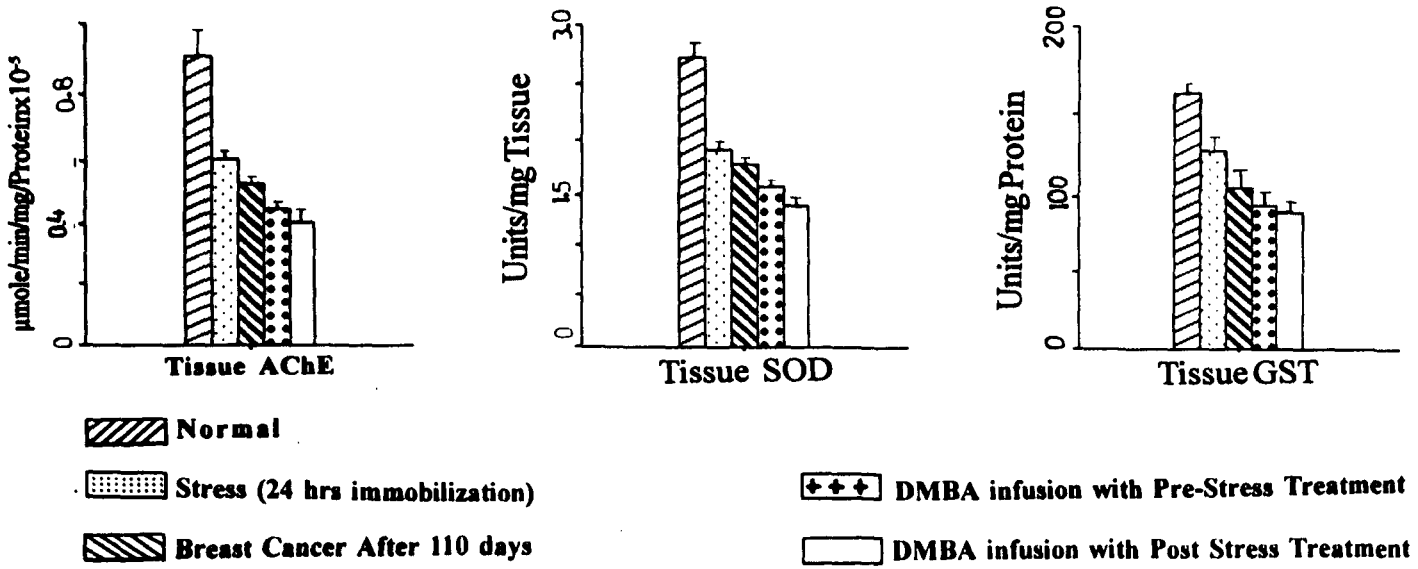


Fig. 36

The brain tissue levels of total, free and protein bound GSH in normal, restraint stressed, DMBA infusion (sacrificed after 110 days) and DMBA infusion with pre and post stress treated rats.

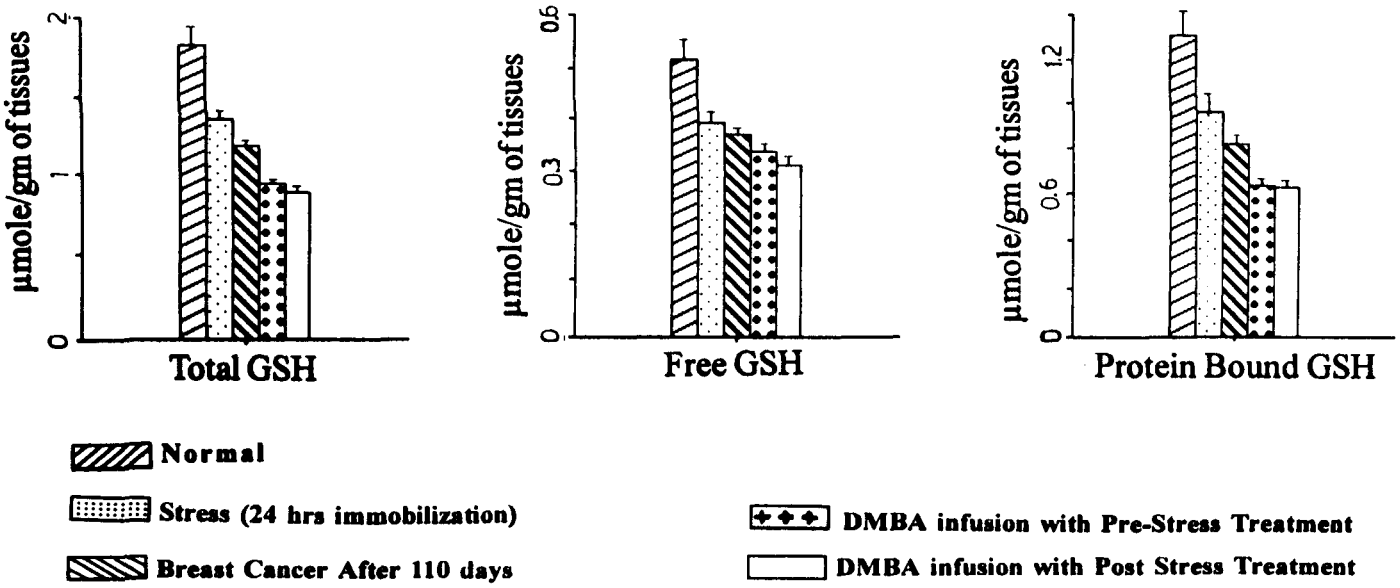


Fig. 37

The spleen tissue levels of AChE, SOD and GST in normal, restraint stressed, DMBA infusion (sacrificed after 110 days) and DMBA infusion with pre and post stress treated rats.

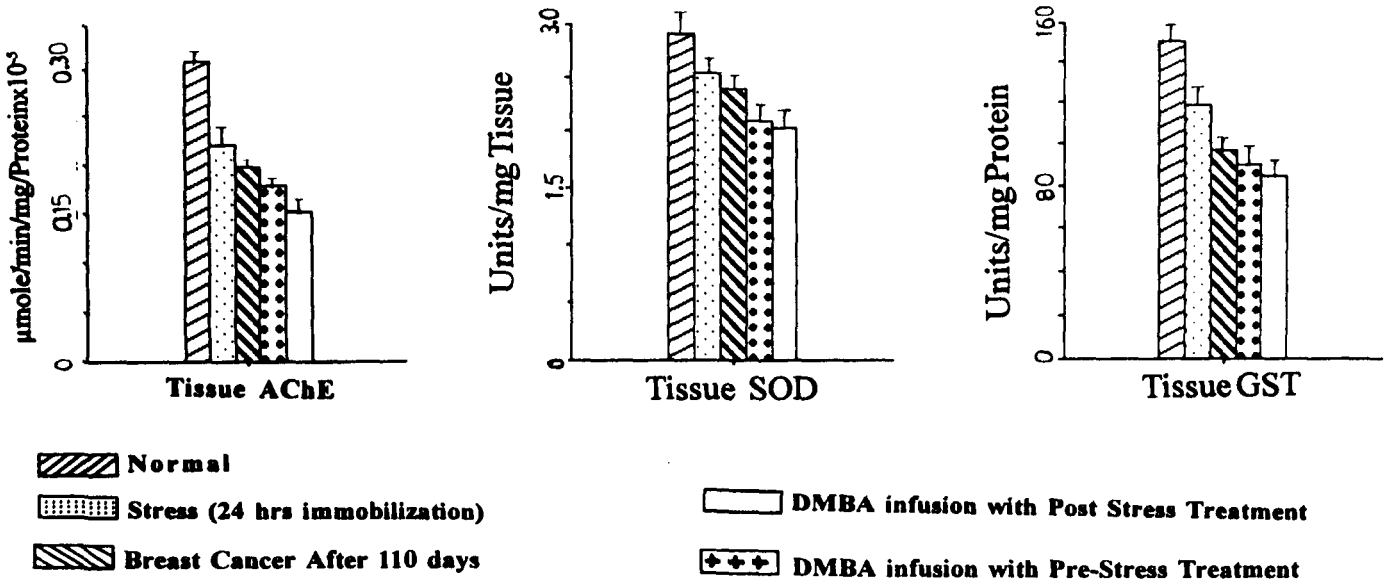


Fig. 38

The levels of total, free and protein bound GSH in spleen tissues of normal, restraint stressed, DMBA indused (sacrificed after 110 days) and DMBA infusion with pre and post stress treated rats.

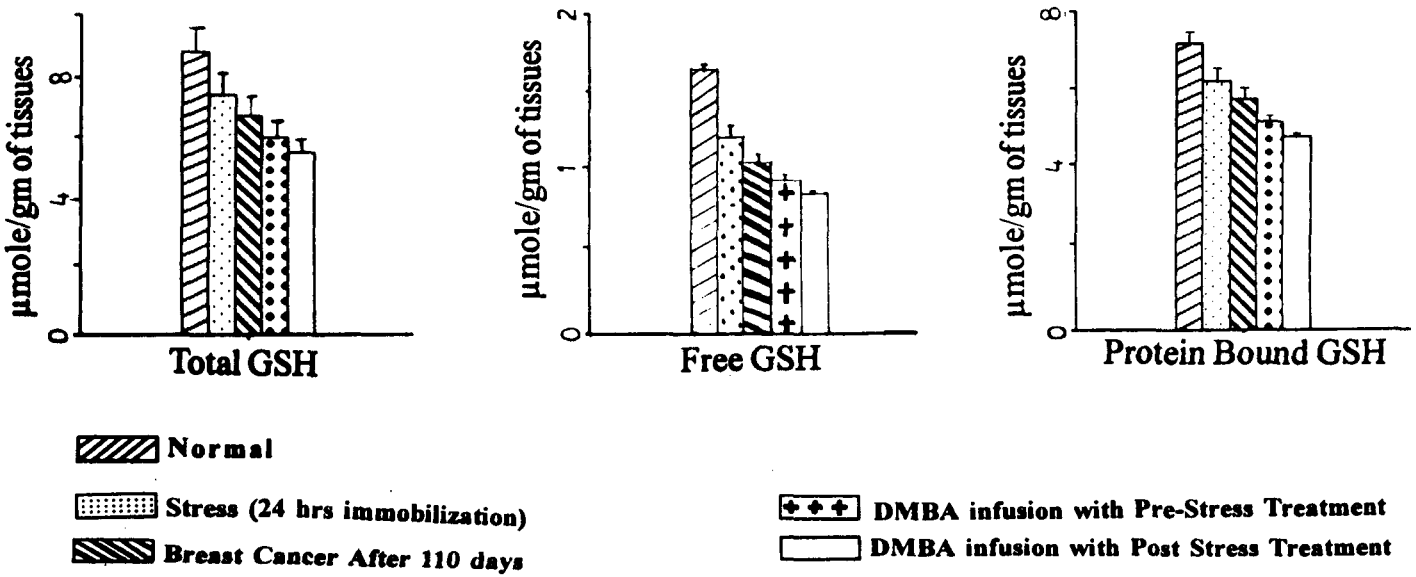


Fig. 39

The circulating activities of AChE and MAO were significantly decreased ($P<0.001$), the levels of cortisol, GOT, GPT and LDH were significantly elevated ($P<0.001$) in DMBA infused post stress treated rats as compared to values obtained from their respective restraint stressed and DMBA only infused rats (Tables 10 and 11, Figs. 28 and 29).

In the tissues of heart, kidney, brain and spleen, the activities of AChE, GST and SOD, the levels of total, free and protein bound GSH were significantly ($P<0.001$ and $P<0.01$)) decreased in DMBA infused post stress treated rats as compared to their respective levels from restraint stressed or DMBA only infused rats. (Table 12-21, Figs. 30-39)

The exposure of rats to stress after DMBA infusion (post stress) had greater effects on the alterations of these biochemical parameters than pre-stress exposure as compared to controls, restraint stressed/or only DMBA infused rats.

Histopathological examinations of liver tissues after various treatment was carried out by pathology Department, J.N. Medical College, A.M.U., Aligarh. Cirrhosis was developed after 24 hours of restraint stress treatment. Both normal hepatic cords and malignant hepatocytes were observed after pre-stress DMBA infusion, while post stress treatment showed only cancerous cells. (Fig. 40-43).

5. Effect of pre and post treatment with Garlic and Salvia on DMBA induced cancer :

If the animals were (pre) treated with garlic and salvia before induction of cancer with the DMBA, the changes recorded in the above

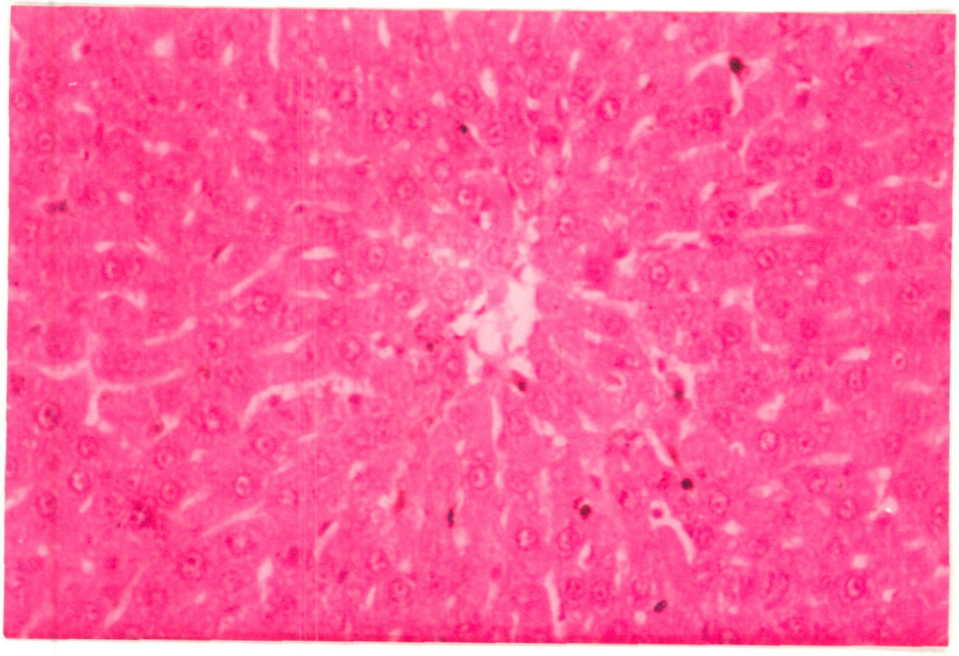


Fig. 40 : Photomicrograph showing normal liver tissue central vein and radiating hepatic cords are seen (Normal rats). (Haematoxylin and Eosin stain x 250)

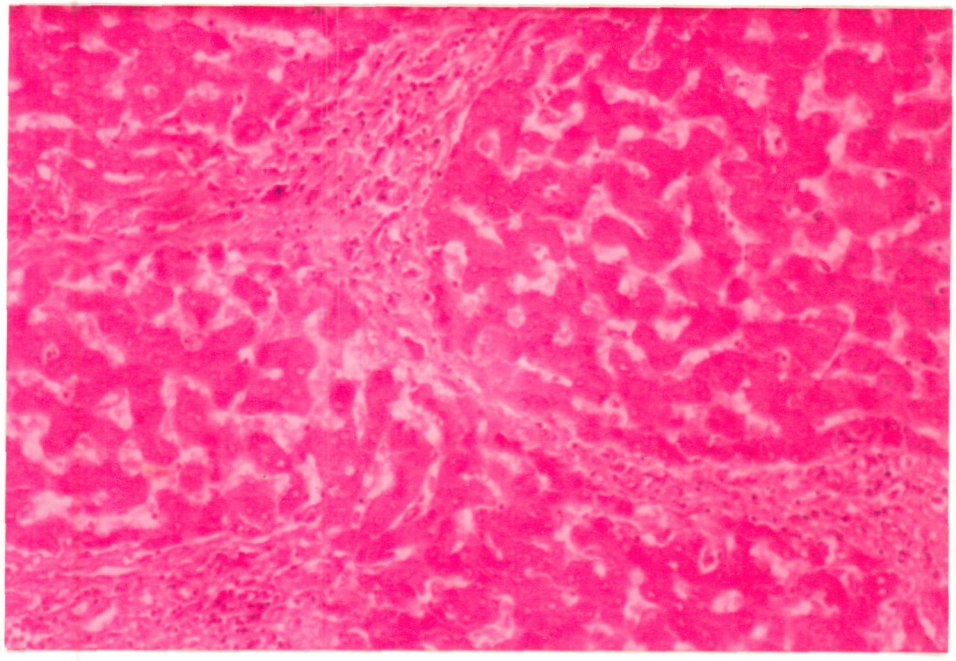


Fig. 41 : Photomicrograph of cirrhosis liver showing pseudolobules of liver cells separated by fibrous bands which are infiltrated by mononuclear cells (24 hours restraint stress treated rats). (H & E x 250)

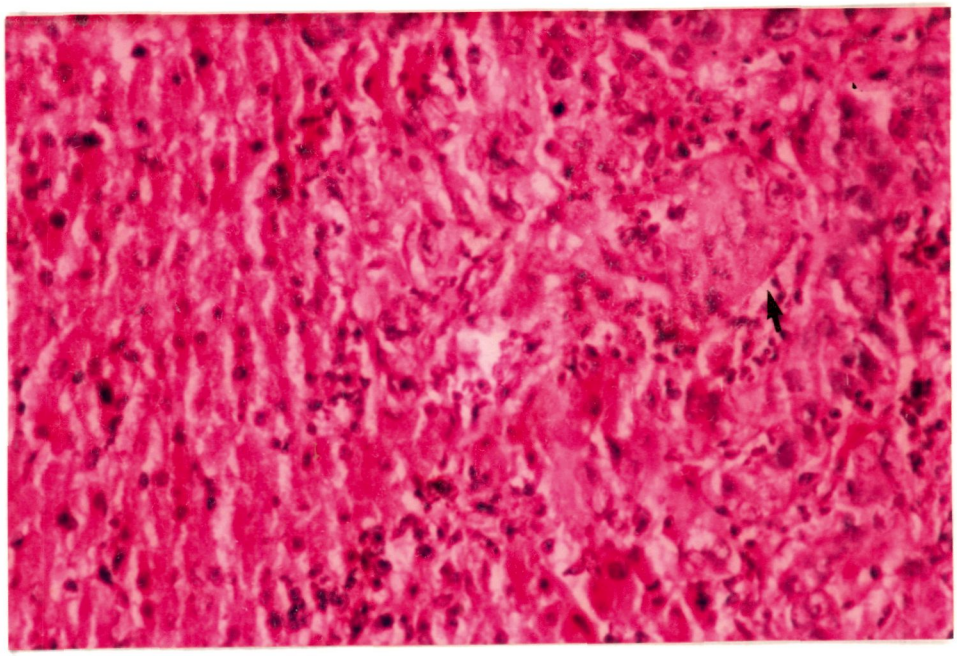


Fig. 42 : Photomicrograph showing normal hepatic cords on the left and malignant hepatocytes on the right. There is marked cellular pleomorphism, atypia and increased mitotic activity; tumour giant cells are also seen (arrow) : (Pre-stress treated DMBA infused cancerous rats).

(H & E x 250)

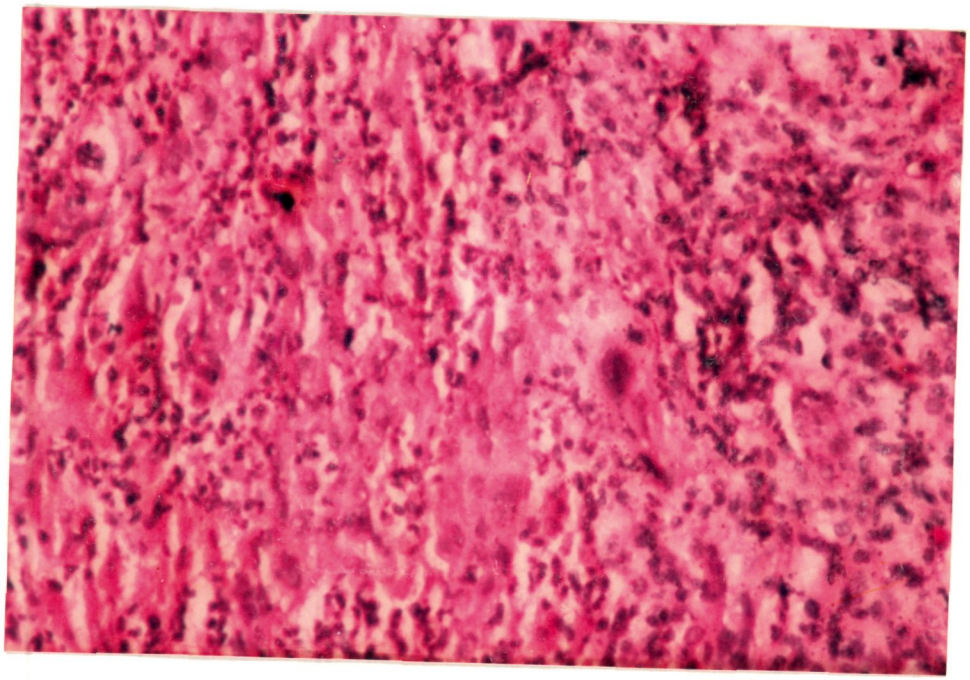


Fig. 43 : Photomicrograph showing hepatocellular carcinoma. The tumour cells have hyperchromatin, enlarged nuclei and exhibit pleomorphism. (Post stress treated DMBA infused cancerous rats).

(H & E x 250).

mentioned biochemcial parameters were less marked than in the untreated animals. The pre treatment with garlic was more effective in preventing the changes in the above biochemical parameters than pre-treatment with salvia. Even though the cancer was developed but the conditions of rats were better than those of untreated DMBA infused cancerous rats (Table 22-45, Figs. 44-67).

Treatment of rats with these drugs after DMBA infusion brought about changes in all the above parameters to bring these a little closer to the values in control animals. With both the drugs (garlic and salvia), post treatment was more effective than the pre treatment. Thus, both drugs can be said to have a better preventive effect on promotion than initiation of DMBA induced cancer so far as the biochemical parameters are concerned. Garlic post-treatment was highly effective than pre-treatment so far as keeping the parameters close to the control value is concerned. Thus, as compared to salvia, garlic had both preventive effects on the initiation and promotion of DMBA induced cancer, while salvia may have promotion effect only (Tables 22-45, Figs. 44-67)

The effectiveness of garlic and saliva on the prevention of initiation and promotion of DMBA induced carcinogenesis can be summarized as follows: Post-treatment with garlic > Pre-treatment with garlic ≥ Post-treatment with salvia > Pre-treatment with salvia.

Osmotic fragility :

The effect of pre and post stress treatment on the hemolysis of RBC membranes from DMBA infused rats.

The stability of RBC membranes in the presence of various

Table - 22

RBCs AChE, Plasma MAO and Cortisol levels in normal, DMBA induced cancer alongwith pre and post garlic treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with	
			Pre-Garlic treatment (10)	Post-Garlic treatment (10)
RBC AChE (µmole/min/mg protein x 10^{-5})	1.380 ±0.095	0.727^{a} ±0.004	$0.886^{aa''}$ ±0.013	$0.957^{ba''}$ ±0.010
Plasma MAO (PU/ml)	2.580 ±0.180	1.650^{a} ±0.023	$2.022^{bb''}$ ±0.073	$2.203^{ba''}$ ±0.085
Plasma Cortisol (µg%)	4.100 ±0.106	7.532^{a} ±0.105	$6.127^{aa''}$ ±0.185	$5.618^{aa''}$ ±0.125

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals.
* a'' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d'' $p<0.1$ as compared to cancer.

RBCs AChE, Plasma MAO and Cortisol levels in normal, DMBA induced cancer along with pre and post Garlic treatment.

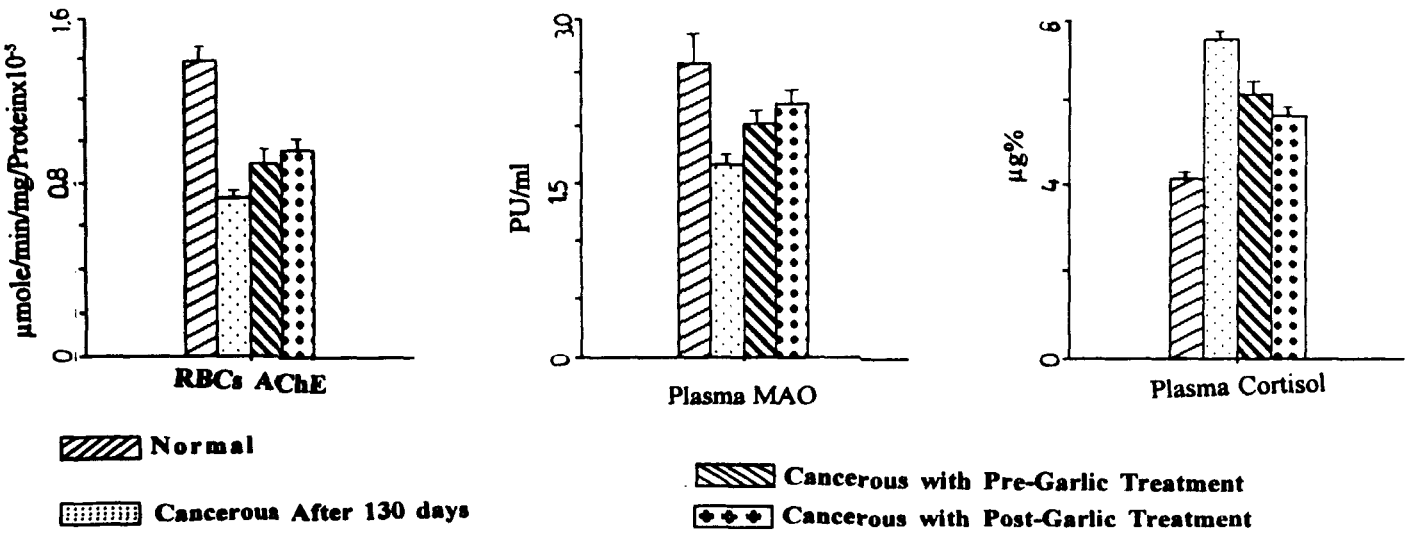


Fig. 44

Table - 23

Effect of pre and post treatment of garlic on serum LDH, GOT and GPT levels in DMBA induced cancerous rats

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
LDH (U/ml)	117.17 ±12.00	189.00[a] ±13.29	160.40[b] ±9.14	141.60[c] ±7.86
SGOT (IU/ml)	13.55 ±1.867	49.26[a] ±1.581	50.85[a] ±1.085	56.06[a] ±1.03
SGPT (IU/ml)	8.86 ±1.195	40.89[a] ±1.453	42.48[a] ±1.011	48.05[a] ±1.450

* The numbers in paranthesis indicate the number of rats.

* a $p<0.001$, b $p<0.01$, c $p<0.05$ in comparison to normals.

Effect of pre and post Garlic treatment on serum LDH, GOT and GPT levels in DMBA induced cancerous rats.

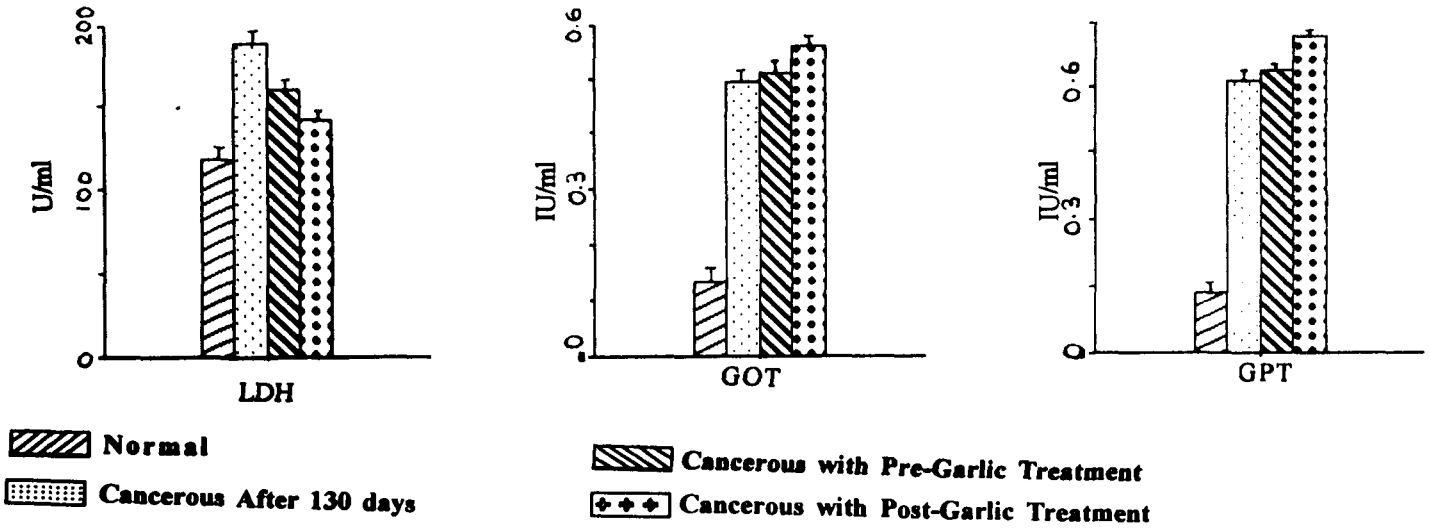


Fig. 45

Table - 24

The rat heart tissue levels of AChE, GST and SOD in the normal, DMBA induced Cancer only and with pre and post garlic treated rats

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Garlic treatment	
			Pre-treated (10)	Post-treated (10)
Tissue AChE (μmole/min/mg proteinx10^{-5})	0.510 ±0.030	0.300^{a} ±0.042	0.362$^{aa''}$ ±0.011	0.388$^{aa''}$ ±0.012
Tissue SOD (Units/mg tissues)	9.270 ±0.139	6.240^{a} ±0.077	7.250$^{aa''}$ ±0.155	8.056$^{aa''}$ ±0.110
Tissue GST (Units/mg protein)	121.40 ±0.850	79.46^{a} ±0.316	100.15$^{aa''}$ ±0.389	109.72$^{aa''}$ ±0.639

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a'' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d' $p<0.1$ in comparison to cancer.

The rat heart tissue levels of AChE, GST and SOD in the normal, DMBA induced cancerous and DMBA induced cancerous rats with pre and post Garlic treatment.

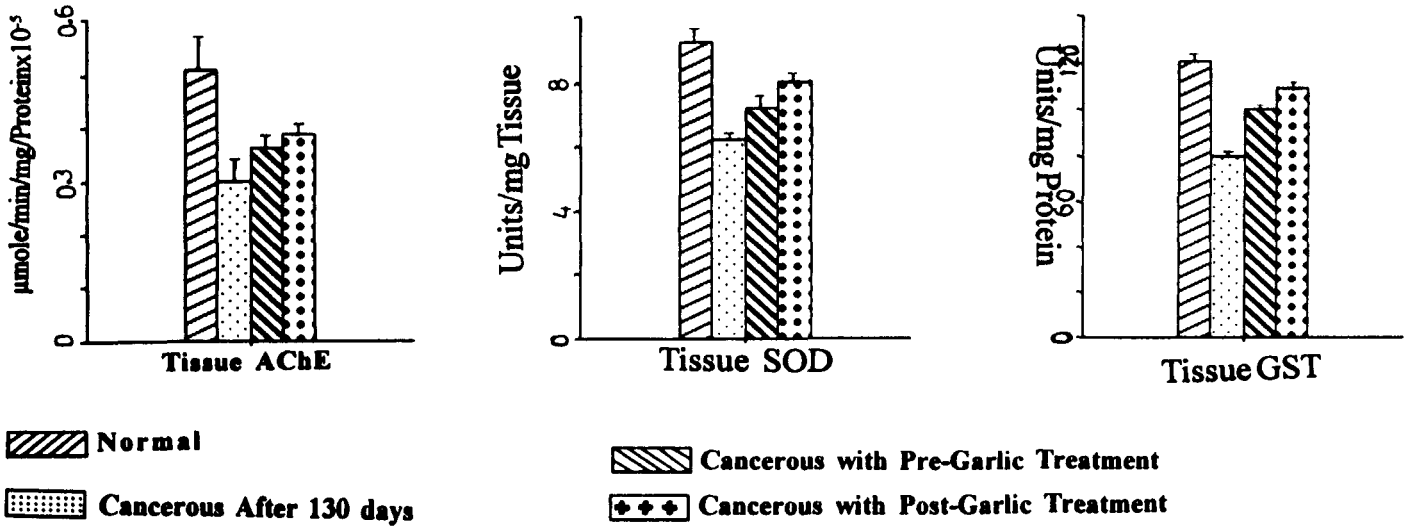


Fig. 46

Table - 25

The levels of total, free and protein bound GSH in normal, DMBA induced cancer and DMBA induced Cancer with pre and post garlic treatment in heart tissues of rat

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Garlic treatment	
			Pre-treated (10)	Post-treated (10)
Total GSH (μ mole/min/gm of tissues)	6.785 ±0.188	4.005[a] ±0.105	4.700[ab"] ±0.0.144	5.775[ca"] ±0.146
Free GSH (μ moles/gm of tissues)	1.298 ±0.043	0.857[a] ±0.014	1.004[aa"] ±0.020	1.109[ba"] ±0.028
Protein bound GSH (μ mole/gm of tissues)	5.349 ±0.230	3.147[a] ±0.090	3.529[ac"] ±0.098	4.665[ca"] ±0.124

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals.
* a" $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ as compared to cancer.

The levels of total, free and protein bound GSH in normal, DMBA induced cancer and DMBA induced cancer with pre and post Garlic treatment in heart tissue of rats.

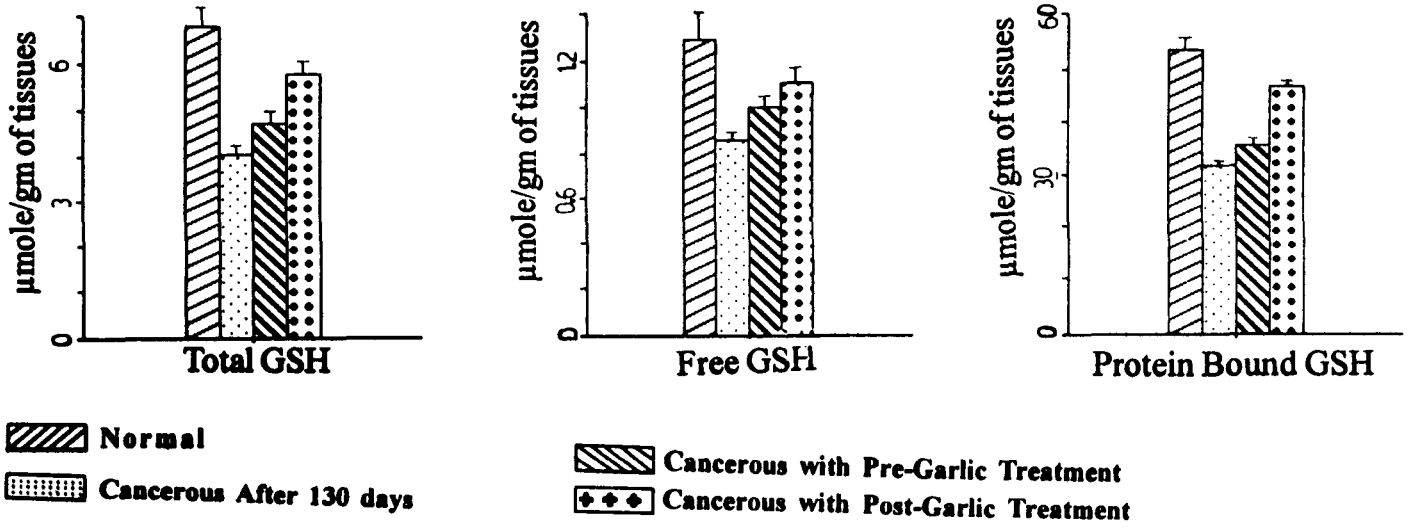


Fig. 47

Table - 26

The levels of AChE, GST and SOD in liver tissues of normal, DMBA induced Cancer only and pre and post gartic treated rats.

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Garlic treatment	
			Pre-treated (10)	Post-treated (10)
AchE (µmole/min/mg protein x 10^{-5})	0.192 ±1.011	0.118[a] ±0.001	0.135[ba''] ±0.0020	0.143[ba''] ±0.002
SOD (Units/mg tissues)	2.640 ±0.111	1.723[a] ±0.026	1.936[aa''] ±0.030	2.233[aa''] ±0.043
GST (Units/mg protein)	169.30 ±1.440	109.30[a] ±0.732	139.06[aa''] ±0.893	151.58[aa''] ±0.639

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b' $p<0.01$, c' $p<0.05$ and d' $p<0.1$ in comparison to cancer.

The levels of AChE, GST and SOD in liver tissues of normal, DMBA induced cancer only and with pre and post Garlic treated rats.

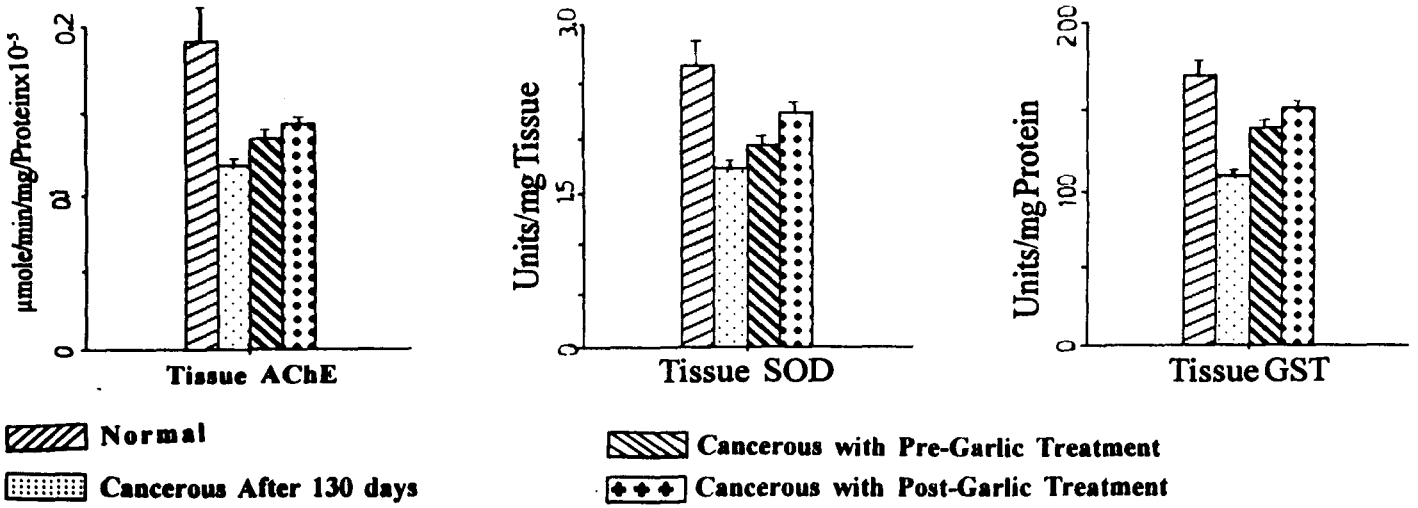


Fig. 48

Table - 27

The liver tissue levels of total, free and protein bound GSH in normal, DMBA induced cancer and cancerous rats with pre and post garlic treated rats

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Garlic treatment	
			Pre-treated (10)	Post-treated (10)
Total GSH (μ mole/min/gms of tissues)	8.613 ±0.264	6.047[a] ±0.245	6.791[ad"] ±0.070	7.265[aa"] ±0.094
Free GSH (μ moles/gm of tissues)	1.480 ±0.061	1.042[a] ±0.037	1.157[ad"] ±0.028	1.255[ab"] ±0.033
Prot.bound GSH (μ mole/gm of tissues)	7.115 ±0.230	5.054[a] ±0.039	5.631[aa"] ±0.059	6.020[aa"] ±0.061

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as comared to normals.
* a' $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ as compared cancer.

The liver tissue levels of total, free and protein bound GSH in normal, DMBA induced cancer only and DMBA induced cancerous rats with pre and post Garlic treatment.

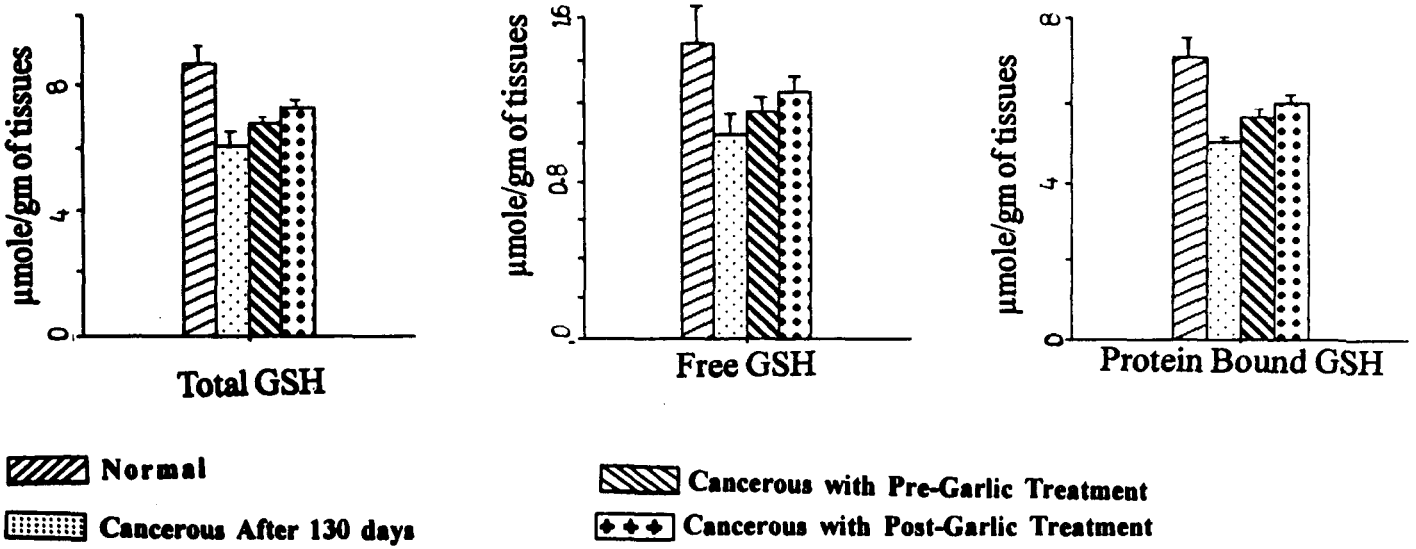


Fig. 49

Table - 28

The Kidney tissue levels of AChE, SOD and GST in normal, DMBA induced cancer only and with pre and post garlic treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Garlic treatment	
			Pre-treated (10)	Post-treated (10)
Tissue AChE (μ mole/min/gm protien x 10^{-5})	0.093 ±0.011	0.057^{a} ±0.001	$0.067^{bb''}$ ±0.002	$0.075^{aa''}$ ±0.003
Tissue SOD (Units/mg tissues)	7.879 ±0.101	5.747^{a} ±0.058	$6.626^{ab''}$ ±0.134	$7.092^{aa''}$ ±0.118
Tissue GST (Units/mg protien)	139.85 ±1.074	89.90^{a} ±0.418	$120.89^{ab''}$ ±0.537	$129.67^{aa''}$ ±0.413

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d'' $p<0.1$ in comparison to stress.

The kidney tissue levels of AChE, SOD and GST in normal, DMBA induced cancer only and with pre and post Garlic treatment.

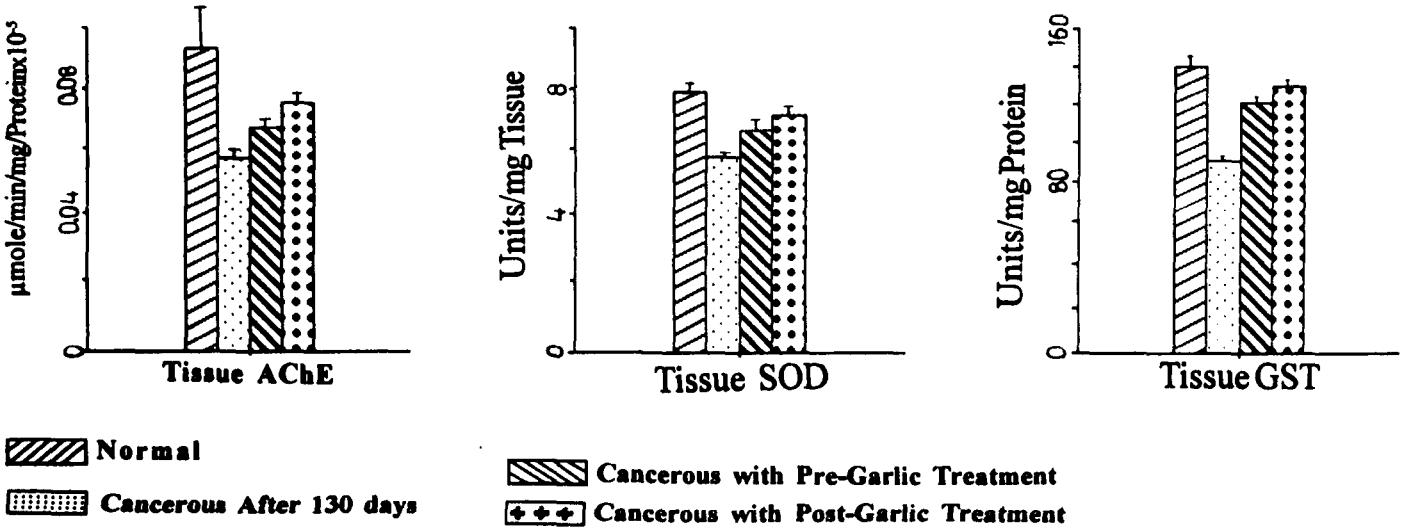


Fig. 50

Table - 29

The levels of total, free and protein bound GSH in normal, DMBA induced cancer and DMBA induced Cancer with pre and post garlic treatment in rat kidney tissues

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Garlic treatment	
			Pre-treated (10)	Post-treated (10)
Total GSH (μ mole/min/gm of tissues)	4.101 ±0.086	2.855[a] ±0.044	3.276[aa''] ±0.042	3.460[aa''] ±0.042
Free GSH (μ moles/gm of tissues)	1.097 ±0.038	0.875[a] ±0.005	0.964[aa''] ±0.013	1.023[ca''] ±0.021
Protein bound GSH (μ mole/gm of tissues)	3.017 ±0.059	1.980[a] ±0.039	2.311[aa''] ±0.029	2.436[aa''] ±0.023

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d'' $p<0.1$ in comparison to cancer.

The levels of total, free and protein bound GSH in normal, DMBA induced cancer and DMBA induced cancerous rats with pre and post Garlic treatment in rat kidney tissues.

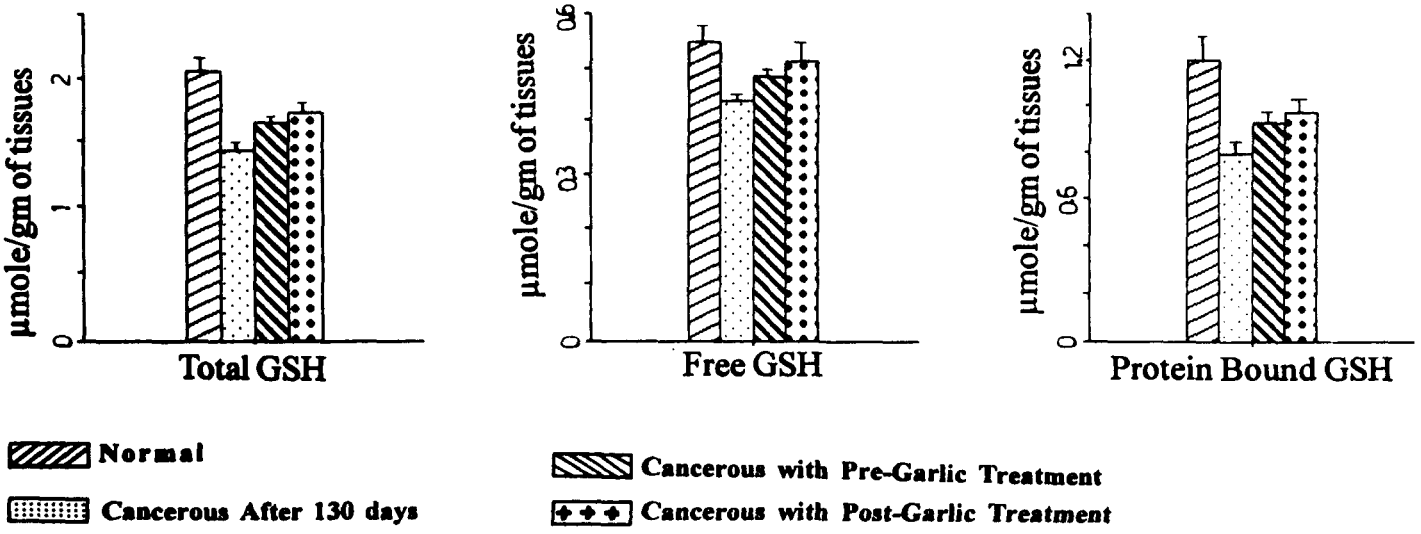


Fig. 51

Table - 30

The brain tissue levels of AChE, GST and SOD in normal, DMBA induced cancer and DMBA induced cancer with pre and post garlic treatment in rats

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Garlic treatment	
			Pre-treated (10)	Post-treated (10)
Tissues AChE (μmole/min/mg protein) x 10^{-5})	0.899 ±0.014	0.500^{a} ±0.008	$0.631^{aa''}$ ±0.021	$0.680^{ba''}$ ±0.043
Tissues SOD (Units/mg tissues)	2.682 ±0.098	1.723^{a} ±0.026	$1.936^{aa''}$ ±0.030	$2.233^{aa''}$ ±0.043
Tissues GST (Units/mg protein)	154.02 ±1.870	99.95^{a} ±1.456	$129.15^{aa''}$ ±0.949	$138.30^{aa''}$ ±0.977

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals.
* a'' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d'' $p<0.1$ as compared to cancer.

The brain tissue levels of AChE, GST and SOD in normal, DMBA induced cancer and DMBA induced cancer with pre and post Garlic treated rats.

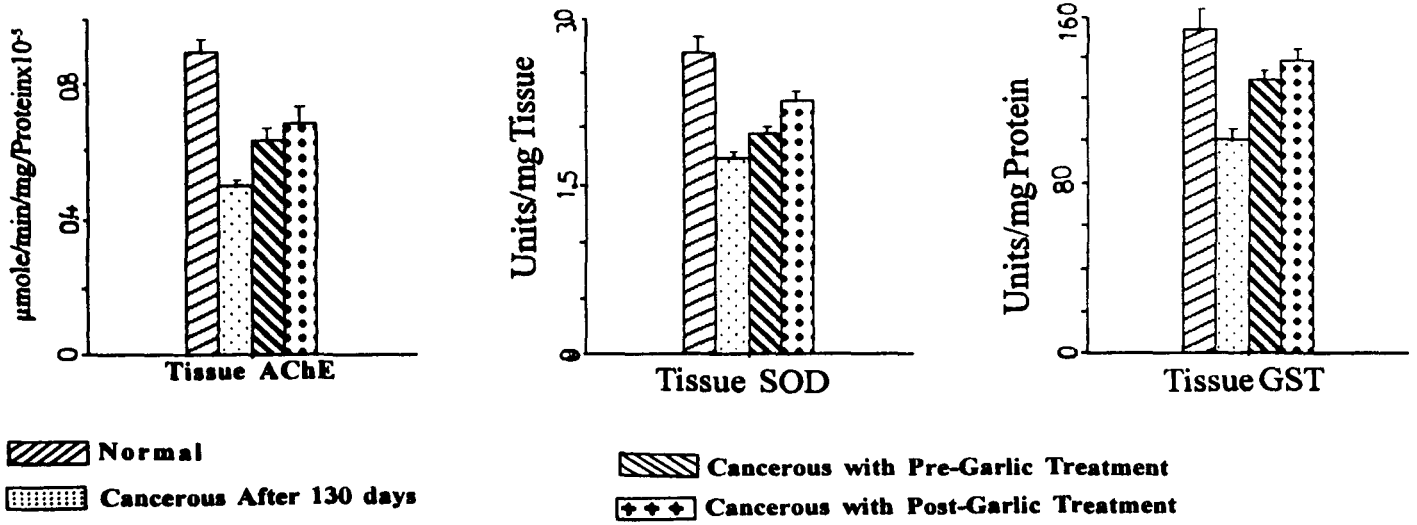


Fig. 52

Table - 31

The levels of total, free and protein bound GSH in normal, DMBA induced cancer and DMBA induced cancer with pre and post garlic treatment in brain tissues

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA Induced Cancer with Garlic treatment	
			Pre-treated (10)	Post-treated (10)
Total GSH (μ mole/min/gm of tissues)	1.910 ±0.060	1.097[a] ±0.024	1.334[aa''] ±0.031	1.642[da''] ±0.039
Free GSH (μ moles/gm of tissues)	0.510 ±0.016	0.353[a] ±0.002	0.384[ad''] ±0.014	0.420[cb''] ±0.020
Protein bound GSH (μ mole/gm of tissues)	1.400 ±0.041	0.746[a] ±0.019	0.949[aa''] ±0.022	1.221[-a''] ±0.027

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d'' $p<0.1$ in comparison to cancer.

The levels of total, free and protein bound GSH in normal, DMBA induced cancer and DMBA induced cancer with pre and post Garlic treatment in brain tissues of rats.

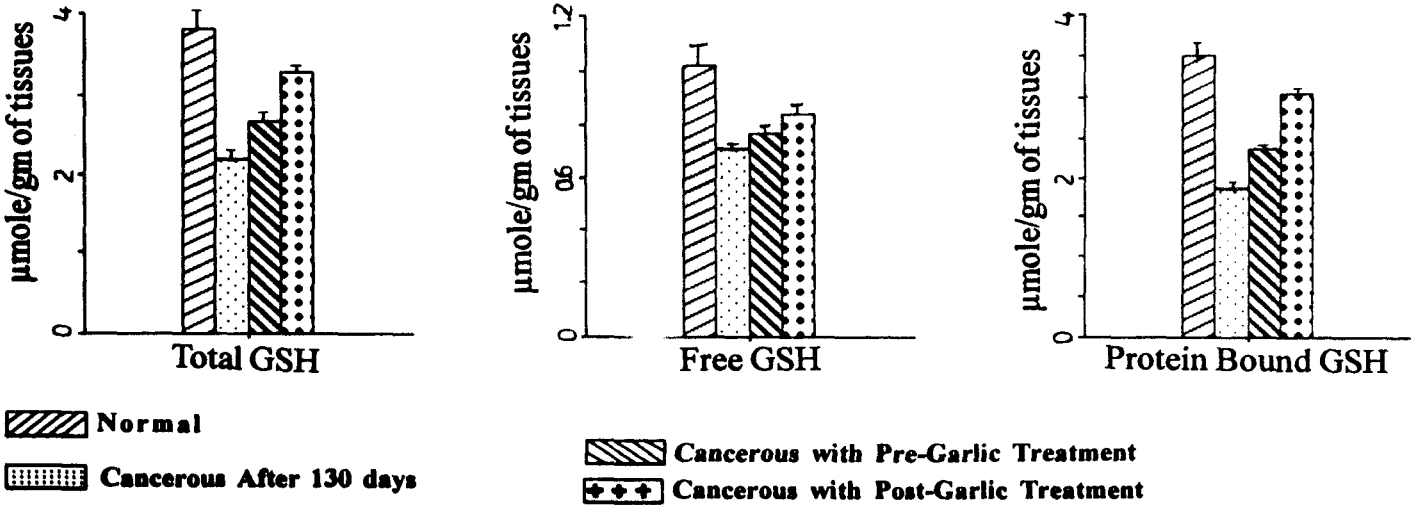


Fig. 53

Table - 32

The spleen tissue levels of AChE, GST and SOD in normal, DMBA induced cancer and DMBA induced cancer with pre and post garlic treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA Induced Cancer with Garlic treatment	
			Pre-treated (10)	Post-treated (10)
Tissues AChE ((μ mole/min/mg protein)x10^{-5})	0.324 ±0.006	0.190^{a} ±0.002	$0.227^{ac''}$ ±0.011	$0.252^{bb''}$ ±0.013
Tissues SOD (Units/mg tissues)	2.885 ±0.005	2.322^{a} ±0.025	$2.554^{ab''}$ ±0.042	$2.707^{Ca''}$ ±0.045
Tissues GST (Units/mg protein)	149.30 ±2.254	91.50^{a} ±0.736	$118.40^{ab''}$ ±0.894	$131.31^{ab''}$ ±0.961

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals.
* a'' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d'' $p<0.1$ as compared to cancer.

The spleen tissue levels of AChE, GST and SOD in normal, DMBA induced cancer only and with pre and post Garlic treated rats.

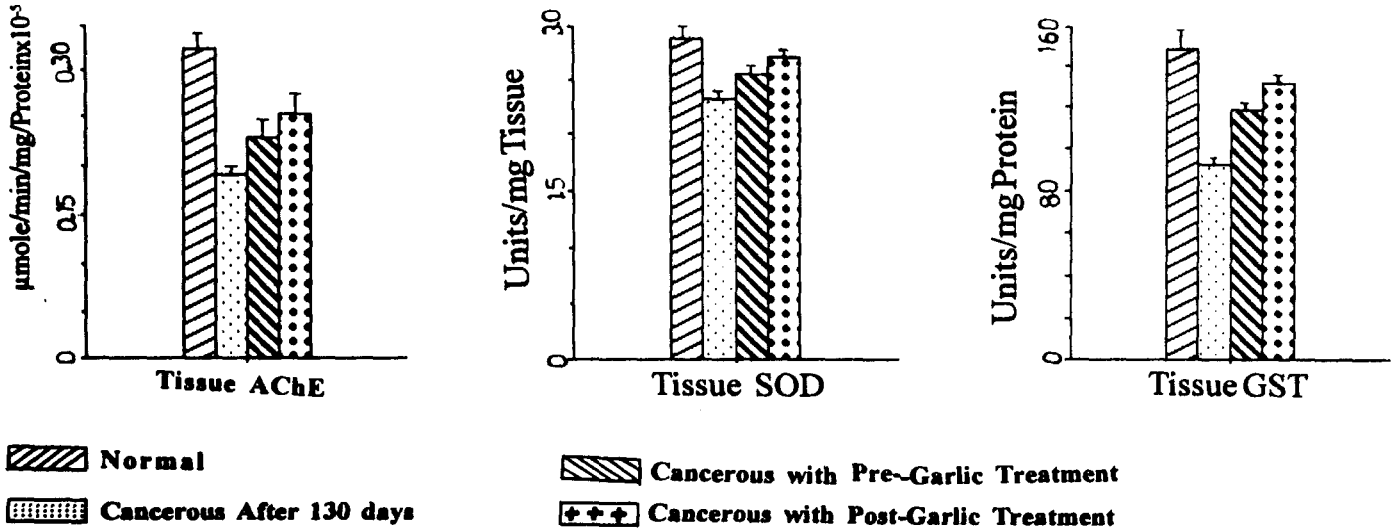


Fig.54

Table - 33

The spleen tissue levels of total, free and protein bound GSH in normal, DMBA induced cancer and DMBA induced Cancer with pre and post garlic treated rats

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Garlic treatment	
			Pre-treated (10)	Post-treated (10)
Total GSH (μ mole/min/gm of tissues)	8.660 ±0.101	6.529[a] ±0.123	7.508[aa"] ±0.093	8.023[ba"] ±0.107
Free GSH (μ moles/gm of tissues)	1.598 ±0.016	0.987[a] ±0.014	1.182[aa"] ±0.024	1.360[aa"] ±0.028
Prot.bound GSH (μ mole/gm of tissues)	7.080 ±0.110	5.537[a] ±0.110	6.373[aa"] ±0.052	6.664[ca"] ±0.079

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a" $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ in comparison to cancer.

The spleen tissue of total, free and protein bound GSH in normal, DMBA induced cancer and DMBA induced cancerous with pre and post Garlic treated rats.

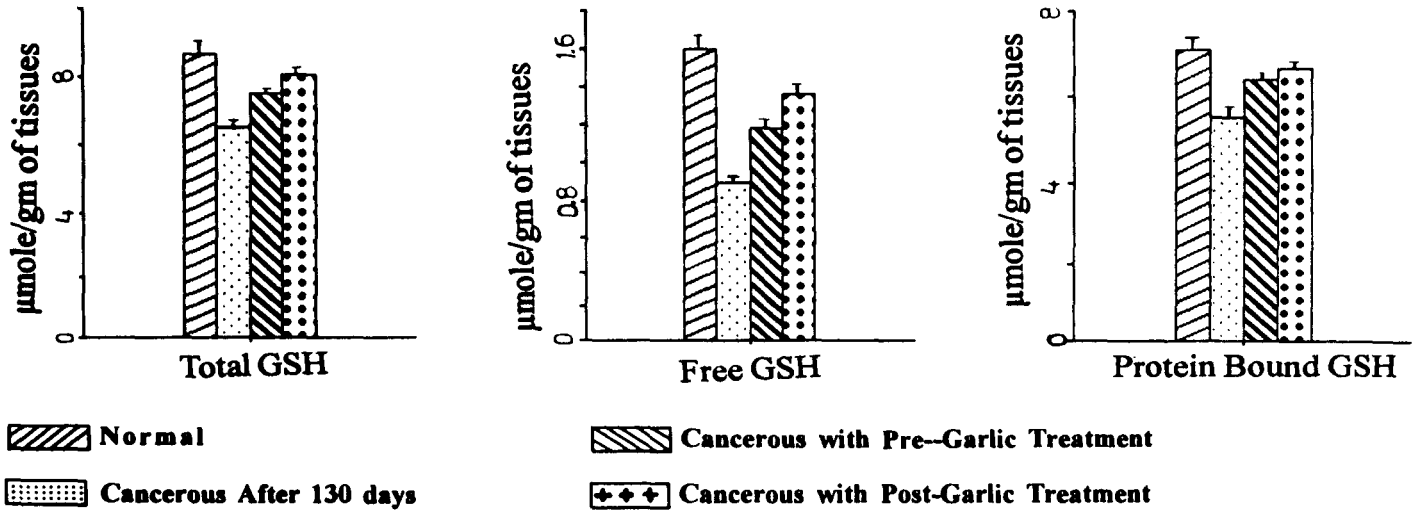


Fig. 55

Table - 34

RBCs AChE, plasma MAO and Cortisol in normal, DMBA induced cancer only and with pre and post salvia treated rats

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
RBCs AChE (μ mole/min/mg protein x 10^{-5})	1.380 ±0.095	0.727[a] ±0.004	0.748[a-] ±0.016	0.867[aa"] ±0.015
Plasma MAO (PU/ml)	2.580 ±0.180	1.650[a] ±0.023	1.707[a-] ±0.085	1.898[ac"] ±0.085
Plasma Cortisol (μg%)	4.100 ±0.106	7.532[a] ±0.105	7.285[a-] ±0.166	6.860[aa"] ±0.120

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a" $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ in comparison to cancer.

RBCs AChE, Plasma MAO and Cortisol in normal, DMBA induced cancer only and with pre and post Salvia treatment.

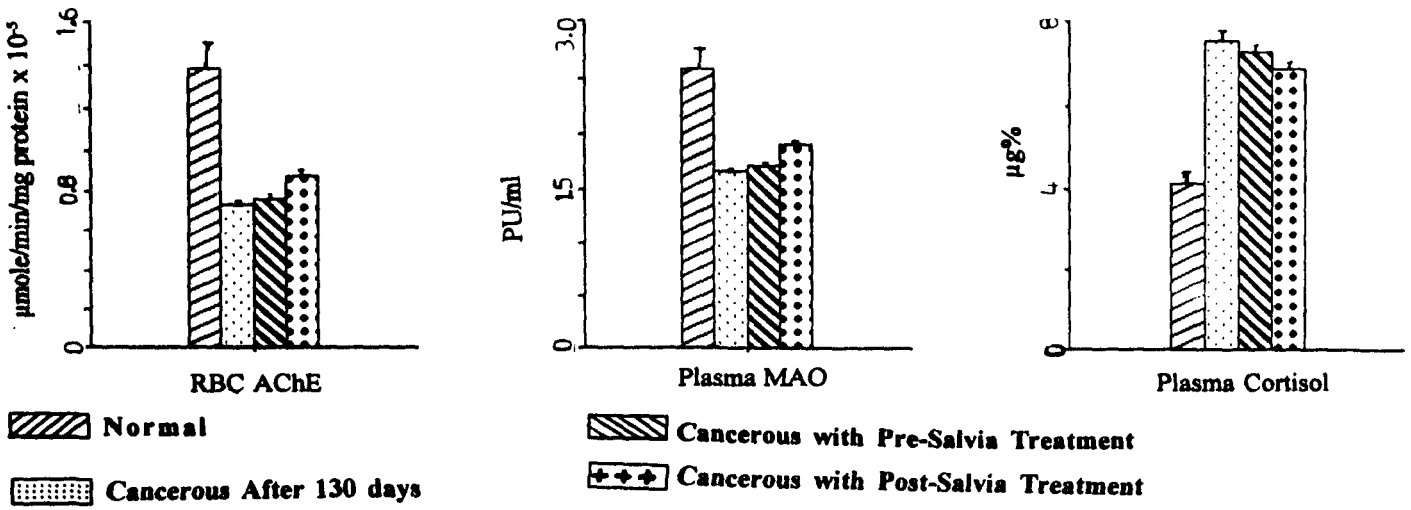


Fig. 56

Table - 35

Effect of pre and post treatment of salvia on serum LDH, GOT and GPT levels in DMBA induced cancerous rats

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
LDH (U/ml)	117.17 ±12.00	189.00[a] ±13.29	181.67[a] ±9.90	163.12[b] ±12.11
SGOT (IU/ml)	13.55 ±1.867	49.26[a] ±1.581	52.00[a] ±1.155	53.45[a] ±1.138
SGPT (IU/ml)	8.86 ±1.195	40.89[a] ±1.453	44.74[a] ±1.691	45.85[a] ±1.380

* The numbers in paranthesis indicate the number of rats.

* a $p<0.001$, b $p<0.01$, c $p<0.05$ in comparison to normals.

Effect of pre and post Salvia treatment on serum LDH, GOT and GPT levels on DMBA induced cancerous rats.

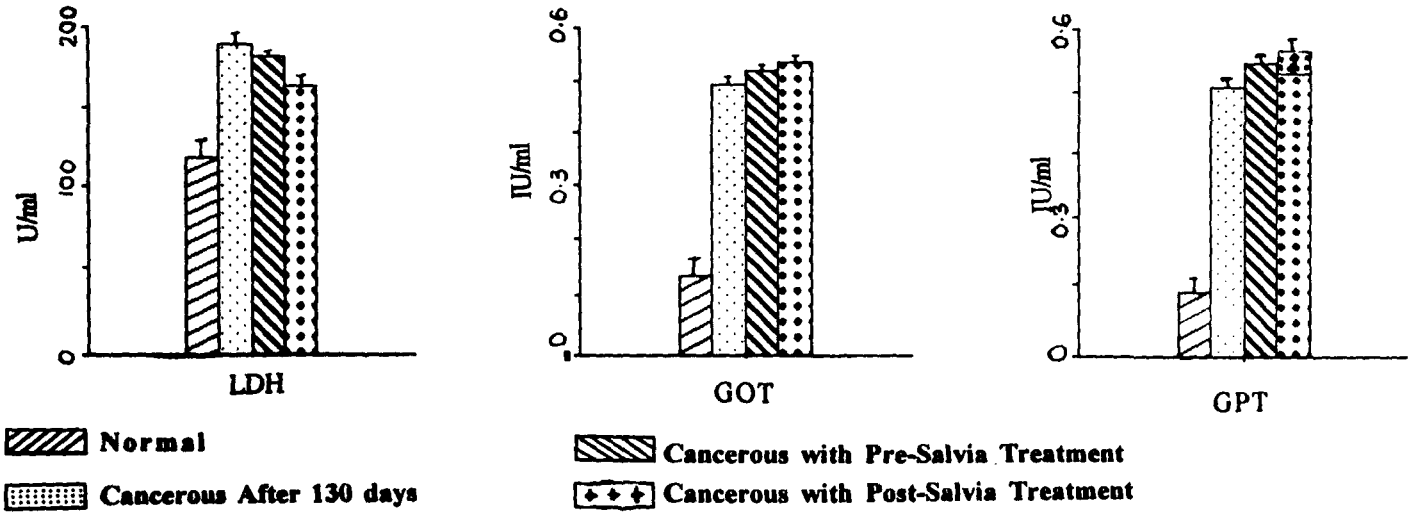


Fig. 57

Table - 36

The heart tissue levels of AChE, GST and SOD in normal, DMBA induced Cancer and DMBA induced cancer with pre and post salvia treaed rats

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
Tissues AChE (μ mole/min/mg protein x 10^{-5})	0.510 ±0.030	0.300[a] ±0.042	0.311[a-] ±0.008	0.356[ab"] ±0.030
Tissues SOD (Units/mg tissues)	9.270 ±0.139	6.240[a] ±0.077	6.493[ac"] ±0.104	7.318[aa"] ±0.104
Tissues GST (Units/mg protein)	121.40 ±0.850	79.46[a] ±0.316	86.66[ac"] ±0.401	91.23[aa"] ±0.593

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a" $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ in comparison to cancer.

The heart tissue levels of AChE, GST and SOD in normal, DMBA induced cancer and DMBA induced cancer with pre and post Salvia treated rats.

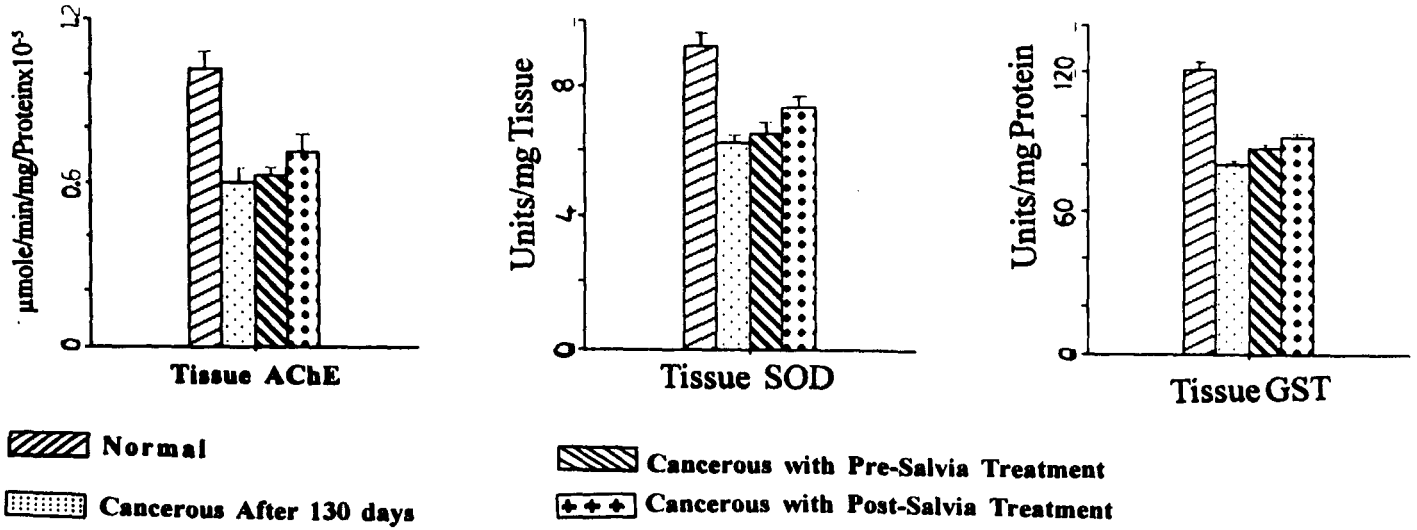


Fig. 58

Table - 37

The heart tissue levels of total, free and protein bound GSH in normal, DMBA induced cancer only and with pre and post salvia treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with salvia treatment	
			Pre-treated (10)	Post-treated (10)
Total GSH (μ mole/min/gm of tissues)	6.785 ±0.188	4.004[a] ±0.105	4.285[ac''] ±0.048	4.647[aa''] ±0.053
Free GSH (μ moles/gm of tissues)	1.298 ±0.043	0.857[a] ±0.014	1.060[ad''] ±0.026	1.195[aa''] ±0.029
Prot.bound GSH (μ mole/gm of tissues)	5.401 ±0.230	3.147[a] ±0.090	3.370[aa''] ±0.144	3.630[aa''] ±040

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as comapred to normals.
* a'' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d'' $p<0.1$ as comapred to cancer.

The heart tissue levels of total, free and protein bound GSH in normal, DMBA induced cancer only and with pre and post Salvia treated rats.

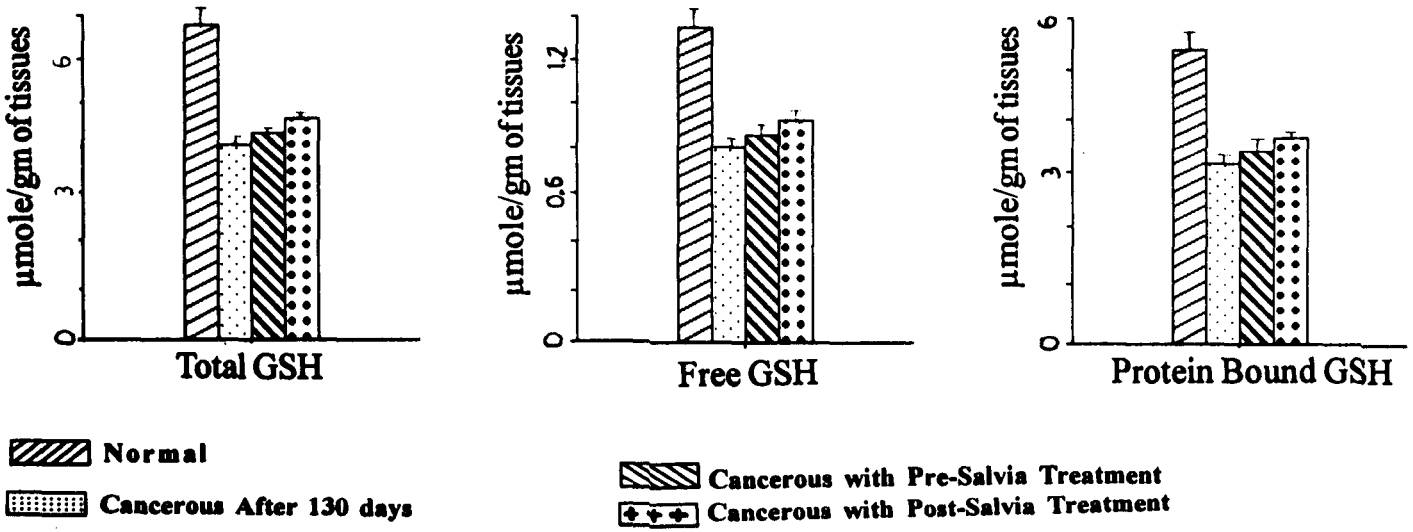


Fig. 59

Table - 38

The levels of AChE, SOD and GST in normal, DMBA induced cancer only and pre and post salvia treated rats in liver tissues

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
Tissues AChE (μ mole/min/mg proteinx 10^{-5})	0.192 ±0.011	0.118^{a} ±0.001	$0.122^{ac''}$ ±0.001	$0.133^{ba''}$ ±0.002
Tissues SOD (Units/mg tissues)	2.640 ±0.111	1.723^{a} ±0.026	1.786^{a-} ±0.021	$1.985^{aa''}$ ±0.040
Tissues GST (Units/mg protein)	169.30 ±1.440	109.30^{a} ±0.732	$120.18^{ad''}$ ±0.833	$127.94^{aa''}$ ±0.809

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals.
* a'' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d'' $p<0.1$ as compared to cancer.

The Liver tissue levels of AChE, SOD and GST in normal, DMBA induced cancer only and with pre and post Salvia treated rats.

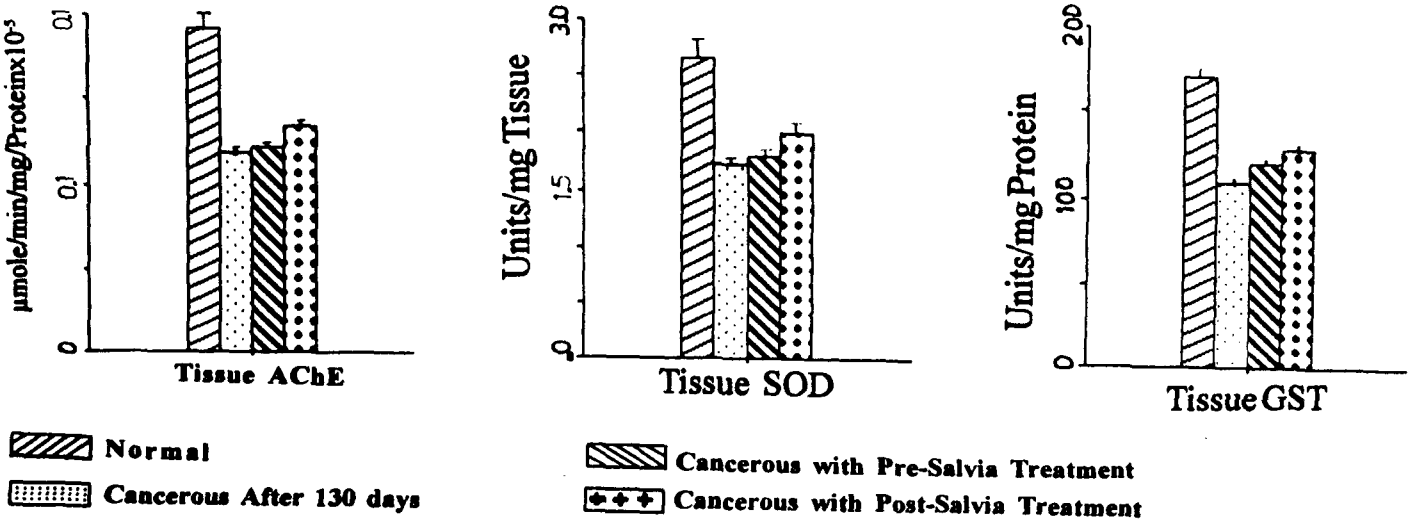


Fig. 60

Table - 39

The liver tissue levels of total, free and protein bound GSH in normal, DMBA induced cancer only and with pre and post salvia treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
Total GSH (μ mole/min/gm of tissues)	8.613 ±0.264	6.047[a] ±0.245	6.145[a-] ±0.131	6.940[ad"] ±0.106
Free GSH (μ moles/gm of tissues)	1.480 ±0.061	1.042[a] ±0.037	1.060[a-] ±0.026	1.195[ab"] ±0.027
Prot.bound GSH (μ mole/gm of tissues)	7.115 ±0.230	5.005[a] ±0.039	5.066[a-] ±0.120	5.742[aa"] ±0.084

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals.
* a" $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ as compared to cancer.

The liver tissue of total, free and protein bound GSH in normal, DMBA induced cancer only and with pre and post Salvia treated rats.

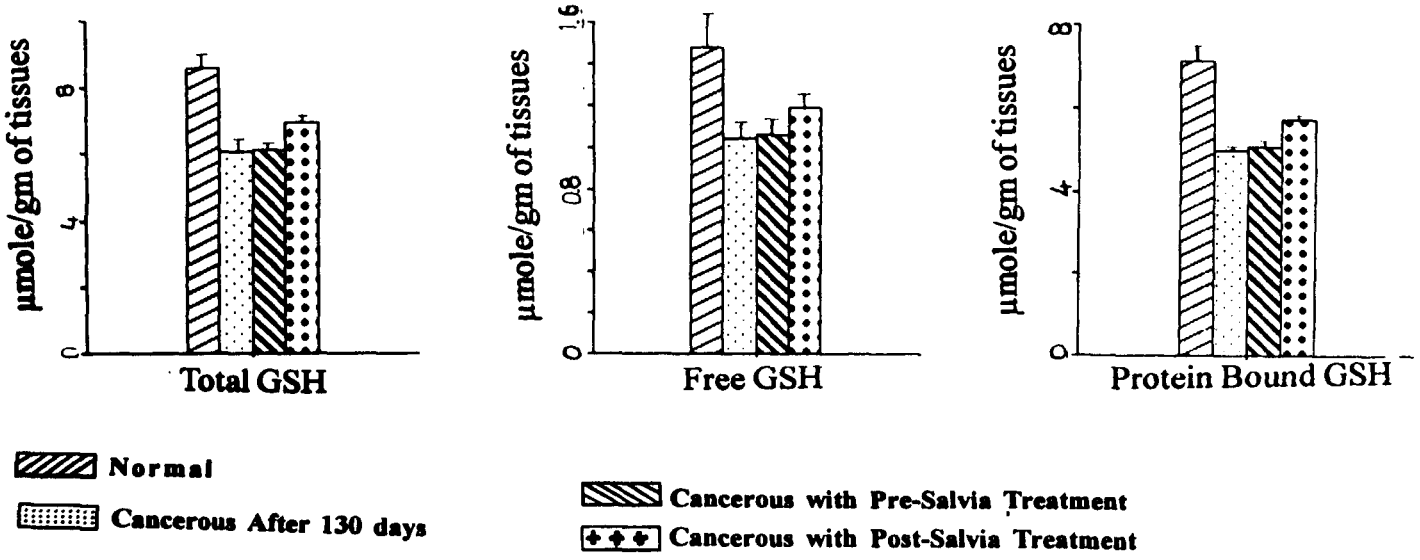


Fig. 61

Table - 40

The kidney tissue levels of AChE, SOD and GST in normal, DMBA induced Cancer only and with pre and post salvia treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA Induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
Tissue AChE (μmole/min/mg protein)x 10^{-5}	0.093 ±0.011	0.057^{a} ±0.0012	0.059^{a-} ±0.001	$0.065^{ba''}$ ±0.001
Tissue SOD Units/mg tissues	7.879 ±0.101	5.747^{a} ±0.058	6.059^{a-} ±0.144	$6.663^{aa''}$ ±0.087
Tissue GST Units/mg protein	139.85 ±1.074	89.90^{a} ±0.418	94.80^{a-} ±0.520	$102.03^{aa''}$ ±0.790

* The number in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a'' $p<0.001$, b'' $p<0.01$, c'' $p<0.05$ and d'' $p<0.1$ in comparison to cancer.

The Kidney tissue levels of AChE, SOD and GST in normal, DMBA induced cancer only and pre and post Salvia treated rats.

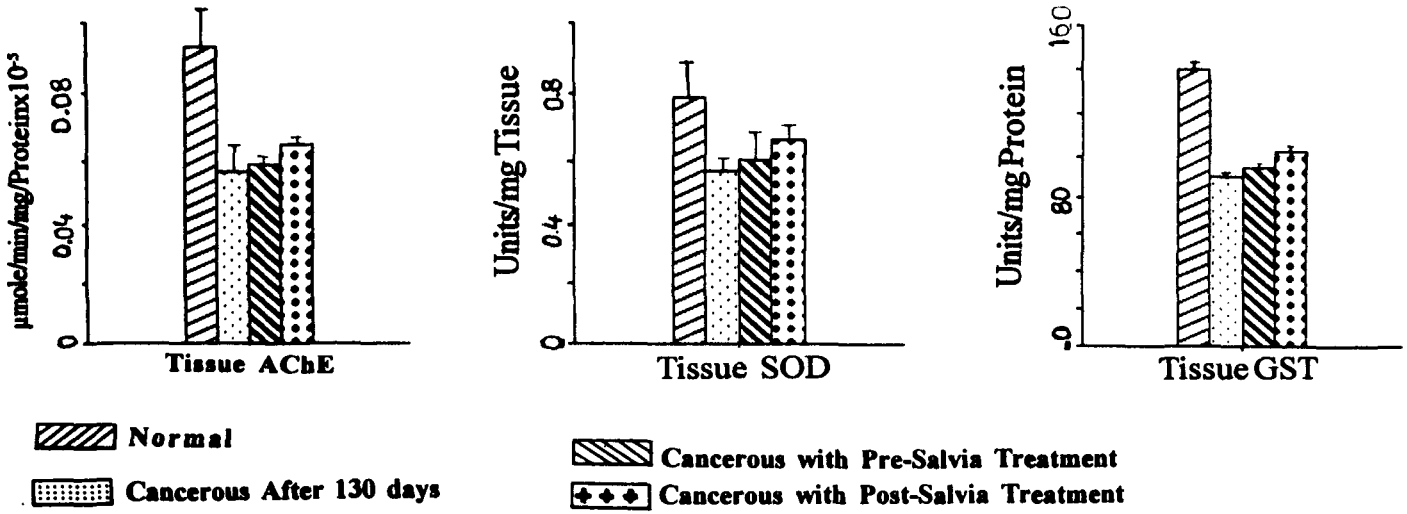


Fig. 62

Table - 41

The kidney tissue level of total, free and protein bound GSH in normal, DMBA induced cancer only and with pre and post salvia treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
Total GSH (μ mole/min/gm of tissues)	4.191 ±0.086	2.855[a] ±0.044	2.988[ad"] ±0.050	3.321[aa"] ±0.051
Free GSH (μ moles/gm of tissues)	1.097 ±0.038	0.875[a] ±0.005	0.903[a-] ±0.013	0.974[bb"] ±0.043
Prot.bound GSH (μ mole/gm of tissues)	3.017 ±0.059	1.980[a] ±0.038	2.085[a-] ±0.037	2.346[aa"] ±0.017

* The number in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals.
* a" $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ as compared to cancer.

The kidney tissue levels of total, free and protein bound GSH in normal, DMBA induced cancer only and with pre and post Salvia treated rats.

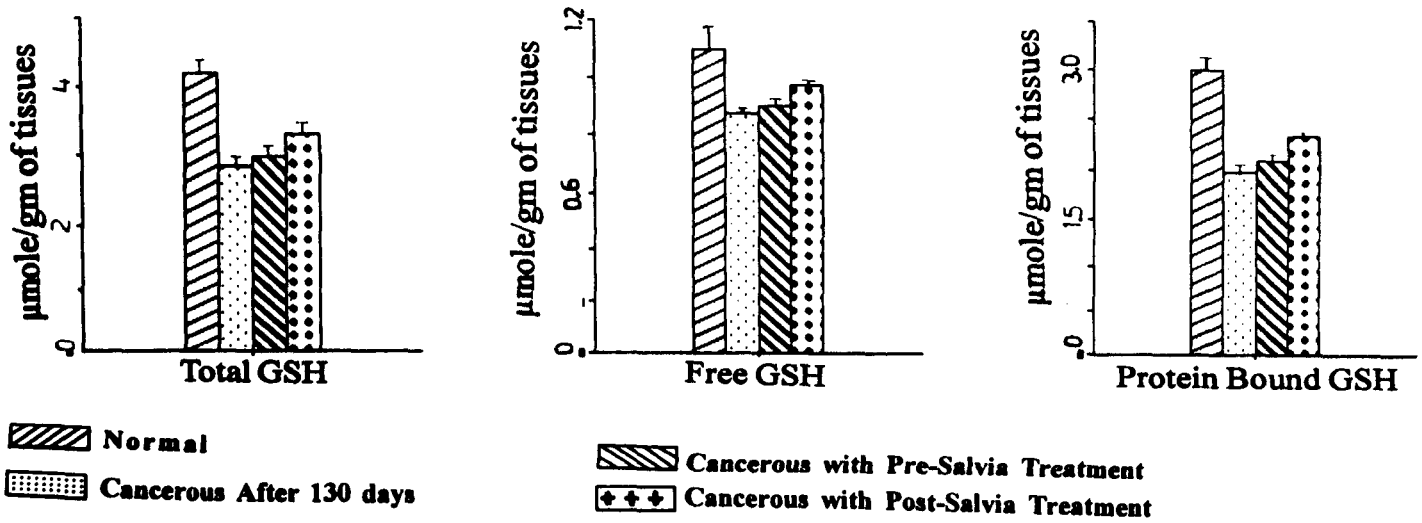


Fig. 63

Table - 42

The brain tissue levels of AChE, SOD and GST in normal, DMBA induced cancer only and with pre and post salvia treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
AChE (μ mole/min/mg proteinx 10^{-5})	0.899 ±0.014	0.500[a] ±0.008	0.551[ac"] ±0.014	0.618[aa"] ±0.022
SOD (Units/mg tissues)	2.682 ±0.098	1.723[a] ±0.026	1.786[ad"] ±0.022	1.985[aa"] ±0.040
GST (Units/mg protein)	154.02 ±1.870	99.95[a] ±1.456	109.67[ad"] ±0.843	116.09[aa"] ±0.876

* The number in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a" $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ in comparison to cancer.

The brain tissue levels of AChE, SOD and GST in normal, DMBA induced cancer only and with pre and post Salvia treated rats.

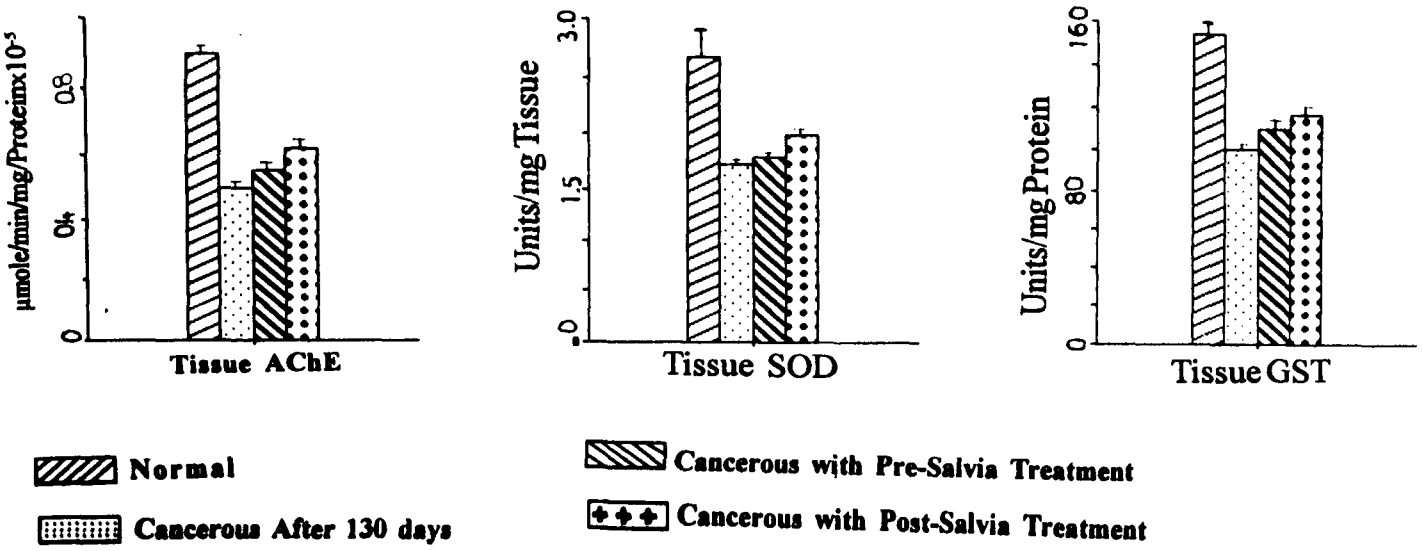


Fig. 64

Table - 43

The levels of total, free and protein bound GSH in brain tissues of normal, DMBA induced cancer and DMBA induced cancer with pre and post salvia treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
Total GSH (μ mole/min/gm of tissues)	1.910 ±0.060	1.097[a] ±0.024	1.242[ab"] ±0.031	1.363[aa"] ±0.034
Free GSH (μ moles/gm of tissues)	0.510 ±0.016	0.353[a] ±0.002	0.356[a-] ±0.012	0.399[bd"] ±0.019
Prot.bound GSH (μ mole/gm of tissues)	1.400 ±0.041	0.746[a] ±0.019	0.886[ab"] ±0.021	0.964[aa"] ±0.018

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ in comparison to normals.
* a" $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ in comparison to cancer.

The levels of total, free and protein bound GSH in brain tissue of normal, DMBA induced cancer and DMBA induced cancer with pre and post Salvia treated rats.

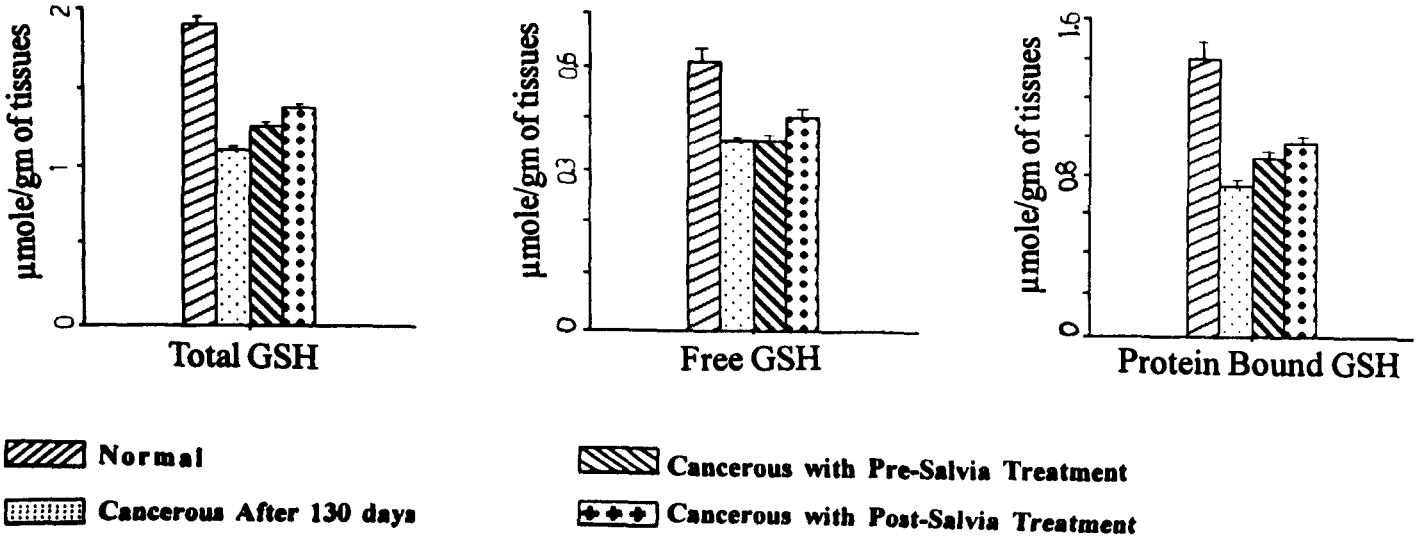


Fig. 65

Table - 44

The spleen tissue levels of AChE, SOD and GST in normal, DMBA induced induced cancer only and with pre and post salvia treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Salvia treatment	
			Pre-treated (10)	Post-treated (10)
Tissue AChE (μ mole/min/mg protein x 10^{-5})	0.324 ±0.006	0.190[a] ±0.002	0.201[ad"] ±0.004	0.228[ab"] ±0.005
Tissue SOD (Units/mg tissues)	2.885 ±0.005	2.322[a] ±0.025	2.390[ad"] ±0.026	2.761[ab"] ±0.027
Tissue GST (Units/mg protein)	149.30 ±2.254	91.50[a] ±0.736	99.65[ad"] ±0.741	116.43[ab"] ±0.710

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals.
* a" $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ as compared to cancer.

The spleen tissue levels of AChE, SOD and GST in normal, DMBA induced cancer only and with pre and post Salvia treated rats.

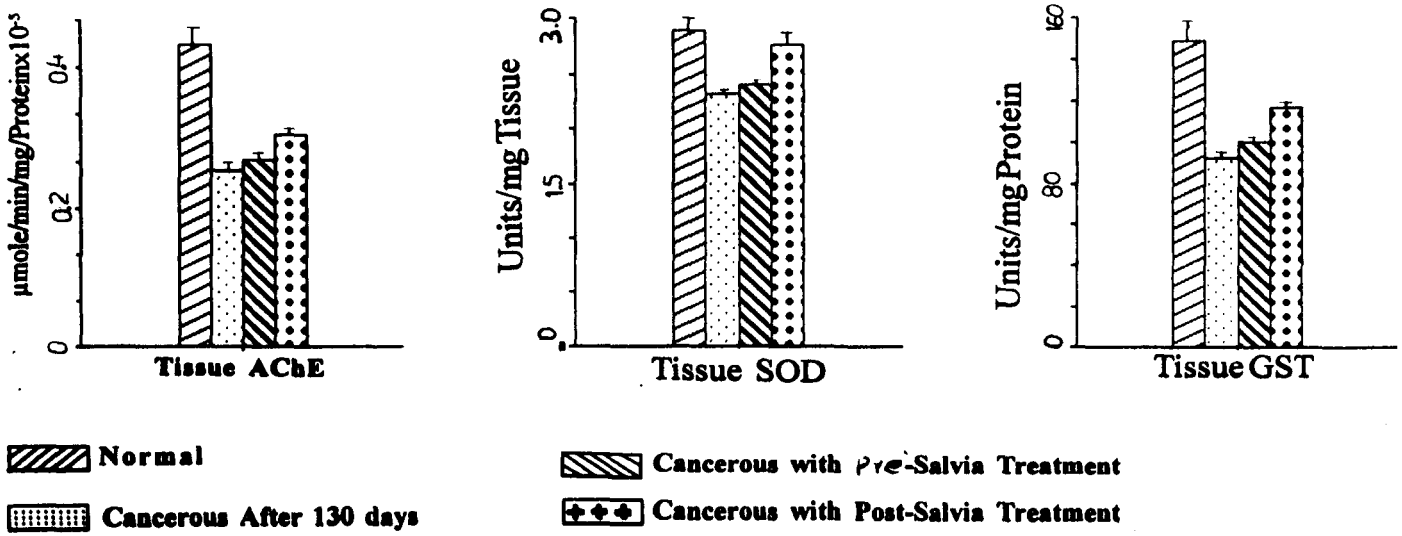


Fig. 66

Table - 45

The levels of total, free and protein bound GSH in the spleen tissues of normal, DMBA induced cancer and DMBA induced cancer with pre and post salvia treatment

(Mean ± S.E.M.)

	Normal (10)	DMBA induced Cancer (10)	DMBA induced Cancer with Saliva treatment	
			Pre-treated (10)	Post-treated (10)
Total GSH (μ mole/min/gm of tissues)	8.660 ±0.101	6.529[a] ±0.123	6.801[a] ±0.094	7.600[aa"] ±0.099
Free GSH (μ moles/gm of tissues)	1.598 ±0.016	0.987[a] ±0.014	1.051[ad"] ±0.026	1.151[aa"] ±0.037
Prot.bound GSH (μ mole/gm of tissues)	7.080 ±0.110	5.537[a] ±0.110	5.784[a-"] ±0.068	6.448[aa"] ±0.064

* The numbers in paranthesis indicate the number of rats.
* a $p<0.001$, b $p<0.01$, c $p<0.05$ and d $p<0.1$ as compared to normals..
* a" $p<0.001$, b" $p<0.01$, c" $p<0.05$ and d" $p<0.1$ as compared to cancer.

The levels of total, free and protein bound GSH in the spleen tissues of normal, DMBA induced cancer and DMBA induced cancer with pre and post Salvia treated rats.

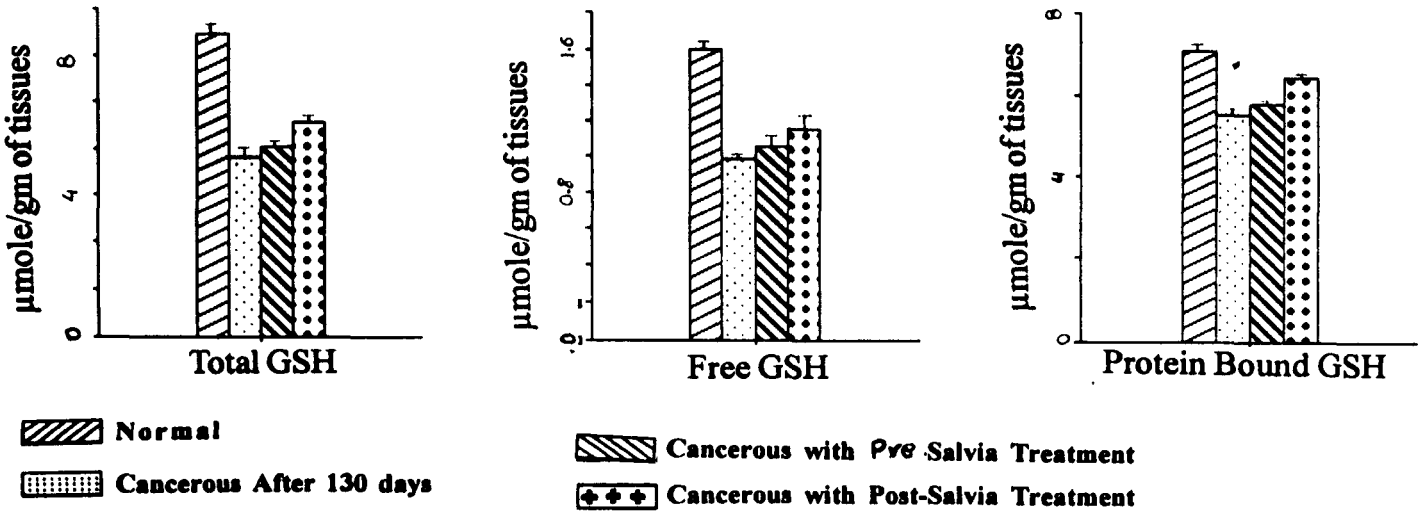


Fig. 67

concentrations of saline was measured in terms of hemolysis of the erythrocyte cells obtained from normal, restraint stressed, DMBA induced cancerous and DMBA infused rats with pre and post stress treatment.

The minimun hemolysis of the erythrocytes was found at 0.9% saline in phosphate buffer irrespective of the treatment given to rats (Table 46 & 47)

The maximum hemolysis of the RBC membranes from stress treated and DMBA infused rats were at 0.4%, and for DMBA infused pre and post restraint stress treated rats were at 0.3 and 0.6% phosphate buffered saline respectively. (Table 46)

The effect of garlic and salvia on RBC membranes hemolysis from DMBA treated rats

The minimum RBC hemolysis was observed at 0.9% PBS for the RBCs from DMBA treated rats with garlic or salvia treatment (pre or post).

The maximum hemolysis of RBC for the DMBA treated rats was at 0.4% of sodium chloride concentration in phosphate buffer. The pre garlic treated DMBA infused rats showed maximum hemolysis of RBC at 0.2%, while post garlic treatment reverted it to normal (0.1%). The RBC of pre and post salvia treated DMBA infused rats had maximum hemolysis at 0.2%. saline in phosphate buffer (Table 47).

Table - 46

Stability of different RBC membranes in the presence of various saline concentrations

RBC Samples (10 each)	Maximum Hemolysis (% saline in PBS)	Minimum Hemolysis (% saline in PBS)
Normal	0.1%	0.9%
Restraint stressed	0.4%	,,
DMBA infused (Cancerous)	0.4%	,,
Pre-stress DMBA treated	0.3%	,,
Post stress DMBA treated	0.6%	,,

Table - 47

Stability of different RBC membranes in the presence of various saline concentrations

RBC Samples (10 each)	Maximum Hemolysis (% saline in PBS)	Minimum Hemolysis (% saline in PBS)
Normal RBCs	0.1 %	0.9%
DMBA infused rats	0.4 %	,,
DMBA infused rats with pre garlic treatment	0.2 %	,,
DMBA infused rats with post garlic treatment	0.1 %	,,
DMBA infused rats with pre salvia treatment	0.2 %	,,
DMBA infused rats with post salvia treatment	0.2 %	,,

The change in weights under various conditions

The rats belonging to each group were weighed on a top Pan balance every ten days after commencement of the experiment and the change in their weight was recorded.

A gradual increase has been recorded in the weight of normal rats from the initiation till the termination of the experiment. In DMBA infused (cancerous) rats, a gradual increase has been observed upto the 80th day, while later on the rats started to lose body weight till 130th day. The weight gain with age was much less by these rats as compared to controls (Table 48, Fig. 68).

The weight gain with age, of the pre-stress treated DMBA infused rats, was much less, rather a loss in weight was recorded till the termination of the experiment as compared to the DMBA infused rats. The weight lost by the DMBA infused post stress treated rats was maximum The physical conditions of the DMBA induced cancerous rats with pre and post stress treatment deteriorated rapidly. The weight loss observed here was rapid with decreased intake of food and increased palpability, cachexia and anorexia. (Table 48, Fig. 68).

The loss in weight after DMBA infusion was reduced by pre or post garlic/salvia treatment. The post garlic treatment was maximally effective in preventing the weight loss due to cancer induction by DMBA (Tables 49 & 50, Fig. 69).

Table No - 48

Alterations in weight of the normal, cancerous, pre and post stress treated DMBA induced cancerous rats during the study (Mean ± SEM)

Groups \ Days	Initial	10	20	30	40	50	60	70	80	90	100	110	120	130
Normal	40.00 ±5.00	50.00 ±6.25	58.00 ±5.25	69.80 3.50	80.20 ±4.10	88.80 6.60	95.40 ±6.80	101.00 ±6.40	105.50 ±5.40	110.00 ±6.20	115.00 ±6.20	118.00 ±6.90	122.00 ±6.10	125.00 ±6.10
DMBA induced cancer	43.00 ±4.50	51.70 ±4.60	55.50 ±5.10	56.50 ±5.40	66.90 ±5.70	78.00 ±5.10	74.00 ±5.80	74.00 ±4.80	84.00 ±5.10	82.50 ±4.40	81.00 ±4.50	80.50 ±4.80	78.00 ±5.00	75.00 ±4.80
DMBA induced cancer with pre-stress treatment	40.00 ±5.00	48.00 ±4.80	55.00 ±5.10	57.00 ±5.50	59.00 ±5.60	71.00 ±5.30	76.00 ±5.50	79.00 ±4.90	75.00 ±5.80	69.00 ±4.10	65.00 ±3.90	62.00 ±4.00	– –	– –
DMBA induced cancer with post-stress treatment	41.00 ±4.90	40.75 ±5.10	32.00 ±4.60	39.00 ±5.40	48.00 ±4.80	57.00 ±4.40	60.00 ±4.70	60.75 ±4.50	58.50 ±4.60	56.00 ±4.60	54.00 ±4.30	– –	– –	– –

Alterations in weight of the normal, cancerous and Pre and Post stress treated DMBA induced cancerous rats

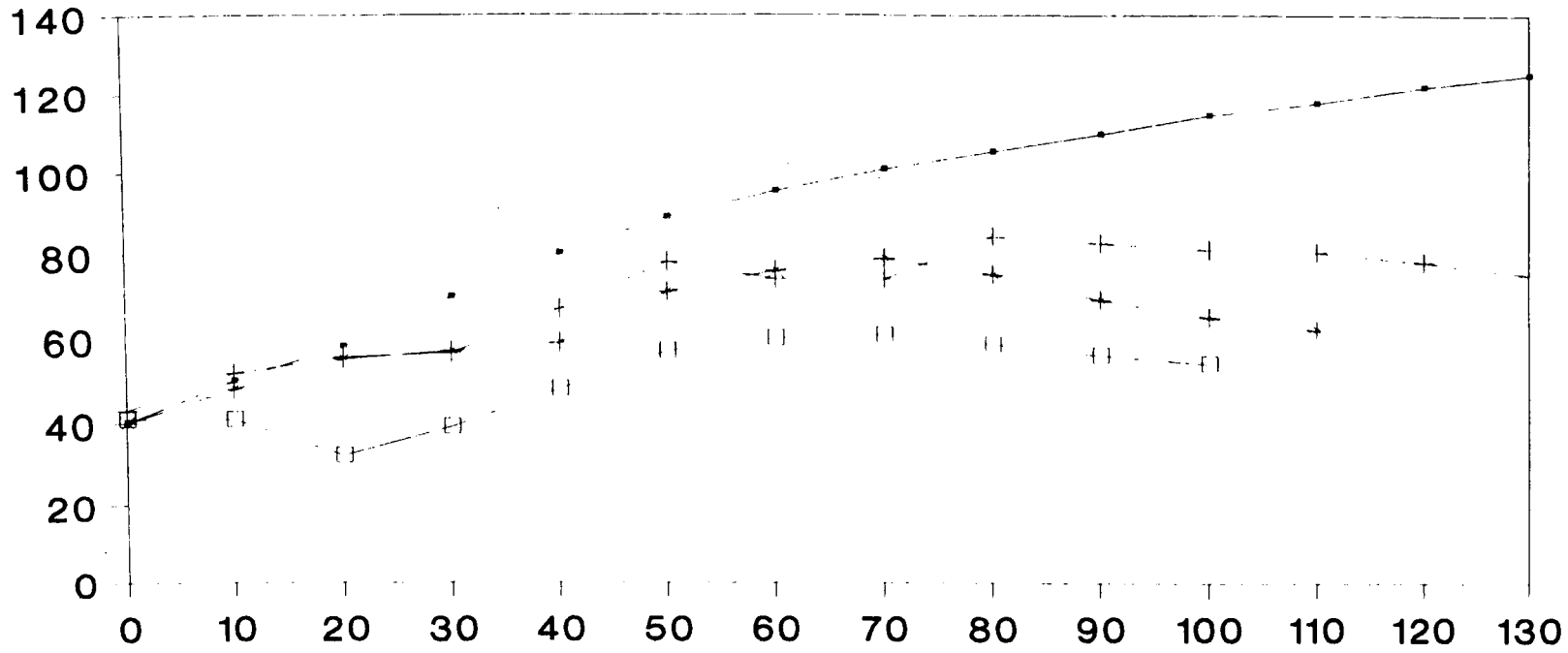


Fig. 68

Normal

DMBA infused rats

Pre-stress cancerous

Post-stress cancero

Table No - 49

Changes in the weights of the normal, cancerous and DMBA infused with pre and post garlic treated rats during the study (Mean ± SEM)

Groups \ Days	Initial	10	20	30	40	50	60	70	80	90	100	110	120	13
Control	40.00 ±5.00	50.00 ±6.25	58.00 ±5.25	69.80 3.50	80.20 ±4.10	88.80 6.60	95.40 ±6.80	101.00 ±6.40	105.50 ±5.40	110.00 ±6.20	115.00 ±6.20	118.00 ±6.90	122.00 ±6.10	125.00 ±6.10
DMBA induced cancer	43.00 ±4.50	51.70 ±4.60	55.50 ±5.10	56.50 ±5.40	66.90 ±5.70	78.00 ±5.10	74.00 ±5.80	74.00 ±4.80	84.00 ±5.10	82.50 ±4.40	81.00 ±4.50	80.50 ±4.80	78.00 ±5.00	75.00 ±4.80
DMBA induced cancer with pre-garlic treatment	43.00 ±5.10	58.00 ±5.40	60.75 ±5.10	60.50 ±3.40	64.88 ±4.60	76.25 ±5.00	76.75 ±4.10	80.00 ±4.40	83.25 ±4.30	90.50 ±4.20	92.50 ±5.00	95.00 ±4.90	98.00 ±4.40	99.50 ±4.30
DMBA induced cancer with post-garlic treatment	40.00 ±5.00	54.25 ±4.90	67.50 ±5.00	78.20 ±4.60	87.50 ±4.80	84.50 ±3.90	88.50 ±4.80	90.70 ±4.90	94.00 ±4.20	98.50 ±5.00	100.00 ±5.90	105.00 ±3.80	106.00 ±4.00	110.50 ±5.40

Table No - 50

Changes in the weights of the normal, cancerous and DMBA infused with pre and post salvia treated rats during the study

(Mean ± SEM)

Groups \ Days	Initial	10	20	30	40	50	60	70	80	90	100	110	120	130
Control	40.00 ±5.00	50.00 ±6.25	58.00 ±5.25	69.80 3.50	80.20 ±4.10	88.80 6.60	95.40 ±6.80	101.00 ±6.40	105.50 ±5.40	110.00 ±6.20	115.00 ±6.20	118.00 ±6.90	122.00 ±6.10	125.00 ±6.10
DMBA induced cancer	43.00 ±4.50	51.70 ±4.60	55.50 ±5.10	56.50 ±5.40	66.90 ±5.70	78.00 ±5.10	74.00 ±5.80	74.00 ±4.80	84.00 ±5.10	82.50 ±4.40	81.00 ±4.50	80.50 ±4.80	78.00 ±5.00	75.00 ±4.80
DMBA induced cancer with pre-salvia treatment	40.00 ±5.10	48.70 ±4.90	53.00 ±5.10	57.75 ±5.60	62.00 ±5.40	67.75 ±5.10	69.50 ±3.50	73.75 ±4.80	78.00 ±4.50	83.00 ±4.80	82.50 ±6.10	92.00 ±4.40	96.00 ±5.50	100 ±5.00
DMBA induced cancer with post-salvia treatment	40.00 ±4.20	53.50 ±4.60	71.00 ±5.10	76.50 ±5.00	80.00 ±4.90	79.50 ±5.10	75.50 ±4.80	78.50 ±5.50	81.50 ±4.80	85.00 ±4.90	90.50 ±5.00	95.00 ±5.00	99.00 ±4.90	104.00 ±5.60

Changes in the weight of DMBA induced cancerous & DMBA infused with pre and post garlic/salvia treated rats

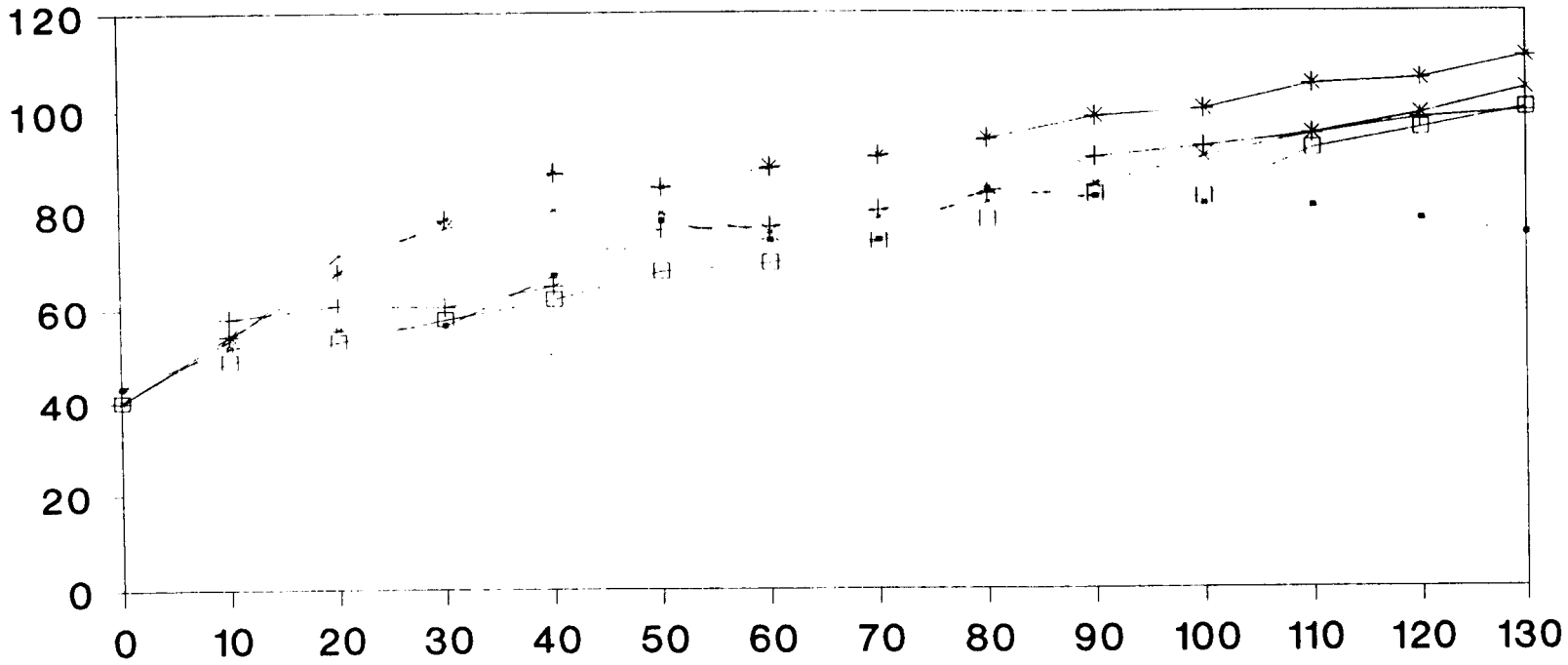


Fig. 69

Discussion

CLINICAL

NORMAL

In the present study, the circulating AChE, MAO, cortisol, LDH, SGOT and SGPT levels were estimated in normal human volunteers of both the sexes. The circulating levels of cortisol and activities of the enzymes AChE and MAO were slightly raised in females than in males, while the serum levels of SGOT, SGPT and LDH were higher in males than in females though the differences were not statistically significant.

Several other workers have reported a slightly raised levels of ACh, cortisol and 5-HT in females (Pandey, 1976; Rai, 1977; Sharma, 1978; Sarkar, 1978 and Patel, 1981), though the differences were not significant. Our findings are also consistent with their observation. A slightly raised level of plasma MAO in females is observed, other workers have demonstrated slightly raised levels of catecholamines in males (Frankenhaeuser *et al.*, 1976). The differences in the biochemical parameters between males and females could be due to the hormonal differences.

ANXIETY NEUROSIS

The circulating levels of cortisol, SGOT, SGPT and LDH were significantly raised, and the activities of AChE and plasma MAO were significantly decreased. All these levels reverted towards their respective control values after treatment (after one month of chemotherapy) in these

patients and were altered less significantly from normals except the levels of SGOT and SGPT which showed a slight insignificant increase after treatment. A significant decrease in AChE activity is observed in experimental animals under stress (Litvak 1969; Seto, 1997). Gupta *et al.* (1978) have reported an enhanced ACh level in psychic stress. The decreased activity of AChE might have contributed in enhancement ACh level.

Estimation of catecholamine has been used as a measure of emotional tension or stress (von Euler, 1964). Alongwith enhanced catecholamine level, raised activity of its synthesizing enzyme is also reported after stress (Kvetnansky, 1972). The decreased MAO activity as observed in the present study might be responsible for enhanced catecholamine levels as observed in stress disorders (Levi, 1963). The activity of MAO is reported decreased in hypophysectomized animals or after hydrocorticosterone treatment (Parvez and Parvez, 1973).

The circulating and urinary levels of corticosteroids are found increased following various stressful stimuli, such as surgical trauma, pain and psychic stress (Thomasson, 1959 and Hume *et al.*, 1962). The involvement of stress is well known in Schizophrenia (Smythies, 1976) and certain other stress disorders (Kulkarni and Kunchandy, 1988). Enhanced levels of corticosteroids have also been reported in variety of diseases (Shimkis, 1961 and Lovezrovement *et al.*, 1955 and Modell, 1997) and experimental studies related to stressful conditions (Von Euler, 1969,

Kvetnansky, 1972; Szentendrei *et al.*, 1980; Reul *et al*, 1997). Similarly a significantly enhanced level of cortisol is observed in anxiety neurotic patients in the present study. The high levels of plasma cortisol may be due to hypothalamic hypophyseal-adrenal stimulation because of anxiety. Twenty percent of the patients on high doses of glucocorticosteroids are found to suffer from depression and psychiatric disorders (Norman, 1998).

An increase in the levels of LDH, SGOT and SGPT is observed in these patients, the levels of LDH reverted towards normal after treatment, while a further increase in SGOT and SGPT levels was observed. Similar to our findings, Pale (1995) has also reported that stress conditions induced a generalized increase in serum enzyme activities of LDH, GOT and GPT and after treatment with antistress drugs, the LDH reverted towards normal values with a slight increase in the GOT and GPT levels. Serum activities of LDH, creatine kinase and aspartate aminotransferase are found enhanced after restraint stress (Sun *et al.*, 1995), which is considered as both physical and psychological stress.

CANCER

CANCER BREAST, CANCER LIVER AND DMBA INDUCED CANCER IN RATS (CLINICAL AND EXPERIMENTAL STUDY)

The trend of changes observed in the levels of biochemical parameters measured are nearly the same in the cancer patients independent of the site of cancer. Thus, the circulating levels of cortisol, GOT, GPT and LDH

were found significantly enhanced, while the activities of RBC AChE and plasma MAO were significantly decreased in cancer liver and breast. After one month of chemotherapy, radiotherapy and surgery all the above biochemical parameters remained significantly altered than the controls in the cancer liver patients, while they reverted to control values in cancer breast patients except the levels of GOT and GPT which showed a significant further rise in cancer liver and a less-significant rise in cancer breast patients after treatment.

In the DMBA infused cancerous rats, the circulating activities of AChE and MAO were significantly decreased, while the levels of cortisol, GOT, GPT and LDH were significantly increased as compared to their respective normals and 24 hours restraint stressed rats. The changes observed in the experimental animals are similar to those of clinical patients.

In the brain, liver, kidney, heart and spleen tissues, the activities of AChE, GST and SOD, the levels of reduced glutathione (total, free and protein bound) were significantly decreased as compared to control values.

The exact cause of malignant disease is not yet known. Various theories have been propounded uptil now, stress is also included as one of the possible factors responsible for oncogenesis (Riley, 1975; Seifter, 1976; Badlwin, 1979, Moore, 1979 and Udupa *et al.*, 1980). Stress is known to enhance the circulating ACh level (Sātio *et al.*, 1976; Gupta *et*

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al., 1978 and Kamysheva, 1978), with a decreased activity of AChE, its degrading enzyme (Litvak 1969). Thus, the enhanced level of ACh as observed in the cancer patients (Banu *et al.*, 1988) may be due to the stress of the disease. Moreover, enhancement of cholinergic receptor have been reported in cancer lung parenchyma (Konaratenko *et al.*, 1995). Cancer being such a dreadful disease, it is quite natural for any person afflicted from it to be under a state of great mental stress and strain. The decreased AChE activity may be responsible for the increased ACh level as seen in cancer patients (Banu *et al.*, 1988). Similar to the clinical finding experimentally induced cancerous rats also showed a decreased AChE activity both in circulation and in various tissues.

Enhanced levels of catecholamines are reported in cases of sarcoma and carcinoma (Udupa *et al.*, 1980, Patel *et al.*, 1981 and Chambrieret *et al.*, 1992). The decreased activity of MAO as observed here in the patients of cancer breast and liver and DMBA infused rats, also suggests an enhanced cathecholamine levels in these cases. The enhanced level of catecholamine produces tissue anoxia and hypoxia through its known vasoconstrictive property. When this situation presists for a long time in a susceptible organ the cells may transform into mutant cells, which can behave abnormally, or mutant cells which are lying dormant may begin to multiply and start a cancer *in situ*. It has been shown by Warburg (1924) also that there is lack of oxygen in cancerous tissues and these cells grow vigrously under anaerobic conditions. Thus, the mutant cells in anaerobic

environment can turn malignant, if such a situation persists for sufficiently long term. The cell undergoing the Warburg effect begins to metabolize sugar by fermentation. As such the cell becomes anaerobic in the presence of adequate oxygen which now becomes toxic (reactive oxygen species) and damages the DNA in chromosomes. Thus, free radicals and reactive oxygen metabolites, due to increased production or reduced inactivation, following a decrease in the antioxidant burden in the mucosa may cause damage to DNA, thereby resulting in genetic alterations. A possible chemopreventive role of antioxidants such as beta-carotene vitamine E and vitamine C has also been a suggested (Pappalarolo, *et al.*, 1996) in oxidative stress. The decreased MAO activity may also be responsible for the increased 5-HT level too. Tryptophan metabolism and hyper-serotonaemia is found associated with breast cancer and carcinoid syndrome (Fahil *et al.*, 1974). A direct correlation between the level of 5-HT and rate of progression of cancer in breast cancer patients has been reported (Banu *et al.*, 1988).

Furth (1975) hypothesized that hormones are not direct carcinogenes but are indispensable components in carcinogenesis, enhancing mutations or unmasking mutations brought about earlier in response to carcinogens, in effect acting as promotors and producing immunosuppression. Several workers have reported a relationship between cancer and preceding stress (Balwin, 1979; Selye, 1979, Cooper and Faragher, 1993 and Anderson *et al.*, 1994). Cortisol is released most

commonly after psychosomatic stress (Hume *et al.*, 1962). Thus, psychological stress helps in the development and progress of cancer possibly by enhancing the release of catecholamine and cortisol from the adrenal medulla and cortex. The enhanced level of cortisol may favour the growth of cancer through its immunosuppressive activity and catecholamines through their vasoconstructive properties by producing tissue anoxia and hypoxia. Kaaja (1996) has also reported that hypoxia in rats produced a proportional loss of body and heart weight with an equal decrease in lactate dehydrogenase units both in heart and muscle tissues. Non-steroidal anti-inflammatory drugs like diclofane and Piroxicam are found to inhibit N-nitrosodiethylamine-induced lung carcinogenesis in mice (Khandija, 1997). The involvement of neurohumoral mechanisms in regulation of lymphocyte function is well known. Moreover, malignancy is associated with alteration of endocrine homeostasis.

The circulating levels of LDH, GOT and GPT have been proved as tumor markers (Vinitha *et al.*, 1995). Serum LDH level has been found enhanced in all the cases of cancers (Ts'O *et al.*, 1996). Two to three fold increase of LDH is associated with fall of plasma cholinestrase level. This relationship is most marked in lymphomas, further fall of plasma cholinestrase level is found to indicate that the disease is fairly advanced and has probably spread to the liver (Ghooi *et al.*, 1980). Our findings confirm the same both by decreased AChE activity and histopathological examination of liver. Cancer patients whose serum LDH was above the

normal range or is not normalized after 3-months of therapy belong to the higher risk group and are prescribed more aggressive treatment (Masukagami *et al.*, 1996). Diamineoxidase (DAO) or histaminase is an enzyme which deaminates histamine and several aliphatic amines to their corresponding aldehydes. Hydrogen peroxide and ammonia are side products of this reaction. It is suggested that the mechanism of action of DAO involves the intermediate generation of superoxide radicals (Silva *et al.*, 1996). Both histamine and histaminase are found enhanced in cancer patients (Banu *et al.*, 1988). This further suggests an enhanced superoxide radical generation in cancer cells. Reactive oxygen species and other free radicals are known to be the mediators of phenotypic and genotypic changes that lead from mutation to neoplasia. The imbalance in tumor cell antioxidant defence mechanism can influence also the sensitivity to cytoreductive therapy. The decreased activities of antioxidant enzymes SOD and GST as seen here and of glutathione peroxidase as observed in multiple myeloma (Zima, *et al.*, 1996) propose a possible role of free radicals in cancer development. However, no relationship is observed between the activities of SOD or glutathione peroxidase and stages of cancer (Zima *et al.*, 1996). While a high serum and pleural fluid SOD activities are reported in patients with squamous cell carcinoma of the lung (Durak *et al.*, 1996). An increased adenosine deaminase and decreased xanthine oxidase, SOD and catalase activities are found in cancerous gall

bladder tissues compared with those of control bladder tissues (Durak *et al.*, 1994) indicating exposure of cancerous tissues to more radicalic stress. Moreover, inhibition of the induction of cancer is observed by various antioxidants (Slaga, 1995). Glutathione which plays a crucial role in the detoxification of xenobiotics is found decreased alongwith reduced GST activity which further aggravates the DMBA induced carcinogenesis in rats. Kurokawa (1997) has also observed depletion of reduced glutathione level in cancer patients.

RESTRAINT STRESS AND ITS EFFECT ON DMBA INDUCED CANCER :

In the present study, the rats were exposed to restraint stress by standardized method (Hasan *et al.*, 1980) for different time intervals as 6,12,18,24 and 30 hours to evaluate the maximum effective period.

The decrease in the activities of RBC AChE and plasma MAO and increase in circulatory levels of cortisol, LDH, GOT and GPT were minimum at 6 and 12 hours as compared to controls, while a gradual rise was recorded at 18 to 24 hours. A maximum change has been observed at 24 hours, while a slight reversion towards normal was seen in the activities of circulatory AChE, MAO, levels of cortisol and LDH with a slight increase in the levels of GOT and GPT at 30 hours of immobilization stress. Thus, in further studies the rats were exposed to 24 hours of stress to evaluate the effect of stress on DMBA induced cancer in terms of above

mentioned biochemical parameters. Mammary cancer was induced by giving DMBA orally (Rogers *et al.*, 1990) as described earlier.

During the 24 hours restraint stress, the activities of RBC AChE and plasma MAO were significantly decreased, while the circulating levels of cortisol, GPT and LDH were significantly elevated. A less significant increase in serum GOT level was observed in the restraint stressed rats as compared to control rats.

The activities of AChE, GST and SOD, the levels of total, free and protein bound GSH were significantly decreased in the tissues (heart, liver, kidney, spleen and brain) of restraint stressed rats as compared to the values obtained from their respective control tissues.

The levels of catecholamines, cortisol and acetylcholine have been reported to be enhanced during stress (Glick *et al.*, 1965; Berson and yelow, 1968; Kvetnansky, 1972; Mikula *et al.*, 1975; Pandey, 1967; Rai, 1976 and Kopin *et al.*, 1980). The oscillation stress is found to deplete brain ACh levels, while restraint stress increases it in experimental animals (Obata and Yamanka, 1994). The decreased activity of AChE in the present study might be responsible for an increase in the ACh level due to its decreased degradations. The activity of AChE is found enhanced in the students with increased body temperature during examination (Yardanova and Gotsa, 1971), while a significant decrease in its activity is observed in experimental animals under stress (Litvak, 1969). During the electric

shock, however, increased levels of both ACh and AChE are reported (Singh *et al.*, 1980). Excercise has been shown to increase the ACh level (Basu *et al.*, 1975 and Weltman, 1994).

The circulating levels of catecholamines and their synthesizing enzymes are found enhanced after stress (Kventansky, 1976), the level of MAO, the catabolizing enzyme of catecholamines is found decreased in the present study which may reflect an enhanced catecholamines level in the restraint stressed rats. The activity of MAO is reported decreased in hypophysectomized animals or after hydrocorticosterone treatment (Parvez and Parvez, 1973). The decreased plasma MAO activity as observed in the present study is consistent with the earlier report of decreased MAO (both A and B) level in immobilization stress (Obata and Yamanaka, 1994). Moreover, acute sympathetic activation by adrenaline infusion, short-term excercise or psychological stress causes a selective increase in circulating natural killer ($CD56^+$) cells than are rich in β-adrenergic receptors (landmann, 1992).

The plasma and urinary 5-HT levels have been found increased after exposure to a variety of stress such as cold stress, immobilization stress, electric shock etc. (Toh, 1960; Sarkar, 1978 and Hirvonan *et al.*, 1978), while some workers have reported an increase in the brain MAO activity after adrenalectomy (Ceasar *et al.*, 1970), and decreased 5-HT level after stress (Corrodi *et al.*, 1968). Moreover, in response to exercise above 60% of VO_2 max, the blood concentrations of a number of stress hormones are

found increased, including adrenline, noradrenaline, growth hormone, β-endorphin and cortisol (Goetz and Pedersen, 1994). Thus, the neurochemical response to stressful stimuli is complex and dependent upon the type of stressor.

The involvement of stress with certain disease conditions is well known, such as in Schizophrenia (Smythies, 1976), and peptic ulcer (Udupa, 1978 and Kulkarni and Kunchandy, 1988), where central 5-HT metabolism is disturbed with the association of decreased platelet monoamine oxidase (Wyatt *et al.*, 1973 and Domino *et al.*, 1976). It has been shown that various stress stimuli are associated with modification of central monoamine metabolism (Subramanian, 1977). MAO inhibitors have been used to treat hypertension and depression but their use is limited due to presence of sympathomimetic amines in foods and drugs (Zellet *et al.*, 1952).

The plasma cortisol levels are found enhanced in various stressful conditions (Von Euler, 1969 and Szentendrici *et al.*, 1980). Similar to our findings, an increase in the circulating and urinary levels of corticosteroids are reported following various stressful stimuli, as surgical trauma, pain anaesthesia, psychic stress and infection (Thomasson, 1959 and Hume *et al.*, 1962). The enhanced cortisol level may be involved in the immunosuppression during stress conditions. Irrespective to the type of stress, the cortisol levels have been reported enhanced (Von Euler, 1969; Kvetnansky, 1972, Mikulaj *et al.*, 1975 and Szentendrci *et al.*, 1980).

The high concentration of glucocorticosteroids suppresses the host immune response. The steroids kill lymphocytes and cause involution of lymphoid tissue. In rodents, glucocorticoids decrease the number of circulating lymphocytes, monocytes and eosiophils, probably by cell lysis. While in humans instead of cell death a shift of the cells occurs from vascular compartment to bone marrow, lymphoid tissue, and spleen (Cupps and Fauci 1982). The rise in plasma cortisol may be due to bodies non-specific response to combat stress.

The circulating levels of GPT and LDH are found elevated significantly, while GOT was less significantly increased in the restraint stressed rats as compared to their respective control values. Increase in serum activity of LDH and aspartate aminotransferase are reported at 2 to 8 hours of immobilization stress (Sun *et al.*, 1995), consistent to the present study, pal (1995) has also reported a generalized increase in serum enzyme activities of GOT and GPT in restraint stress. The antistress activity of N-phthaloyt-gamma-aminobutyric acid in rats is found to normalize restraint stress induced increase of LDH, but causes a further enhancement of GOT and GPT activities (Vinitha *et al.*, 1995). The enhanced LDH level may reflect its secretion into the plasma from RBC due to free radical induced damage of RBC membranes as free radical generation is seen enhanced during stress (Zima *et al.*, 1996).

At 24 hours of immobilization, the activities of AChE, GST and SOD, levels of total, free and protein bound GSH were significantly

decreased in the tissues of heart, liver, kidney, spleen and brain as compared to values obtained from tissues of their respective normal rats. Changes in the tissues concentrations of GSH or GSSG may be either reciprocal or involve alterations in the glutathione pool. The enzymatic mechanisms that lead to synthesis of glutathione or to its reduction have recently been described in some details (Meister, 1974; Orlowski and Karkowsky, 1976). GSH concentration is observed decreased after 30 minute of complete Ischemia (Folbergroe *et al.*, 1979). The decrease in the GSH level is not found to correspond with an increase in the GSSG concentrations reflecting anaerobic break down of GSH (Falbergrova *et al.*, 1979) . Other workers have also reported reduced brain tissues concentration of GSH during mild hypoxia in rats (Wideman and Domanska, 1974 and Jarostava, 1979).

Glutathione in brain and other tissues is considered to have important function in protecting cells against oxidative damage (Orlauski and Karkowsky, 1976) acting as a free radical scavanger (Rink, 1974 and Jaroslava, 1979). Thus, glutathione as a biological antioxidant has a role in destruction of free radical and protection of cells during aging too (Pruche *et al.*, 1991). Immobilization stress induces generation of reactive oxygen species and decreases the endogenous antioxidant defenses, which can be attenuated by extra cellular administration of antioxidant GSH (Liu *et al.*, 1994). The depletion of glutathione during immobilization stress stimulates oxidants and causes oxidative damage resulting into

degenerative diseases of aging including brain dysfunction (Liu *et al.*, 1996). The pathogenesis of parkinson's disease is also related to the decrease of antioxidant glutathione (Drukarch *et al.*, 1996), as glutathione is essential for the repair process in brain exposed to oxidative damage by free radicals (Pellmur, 1992). Thus, the reduced GSH level with a reduced activity of both SOD and GST further aggravates the situation caused by reactive oxygen species. The effect of stress on the intiation or promotion of DMBA induced carcinogenesis was evaluated in terms of above mentioned biochemical parameters.

Effect of stress on DMBA induced cancer in rats :

The effect of stress on initiation and promotion of DMBA induced carcinogenesis was evaluated in terms of afore mentioned biochemical parameters. For the study each rat was immobilized for 24 hours (showing maximum stress effect) prior and after a single dose of DMBA (30 mg/kg body weight, by gavage) as described earlier. The biochemical parameters were assayed and compared with both stressed (alone) and DMBA (alone) infused rats.

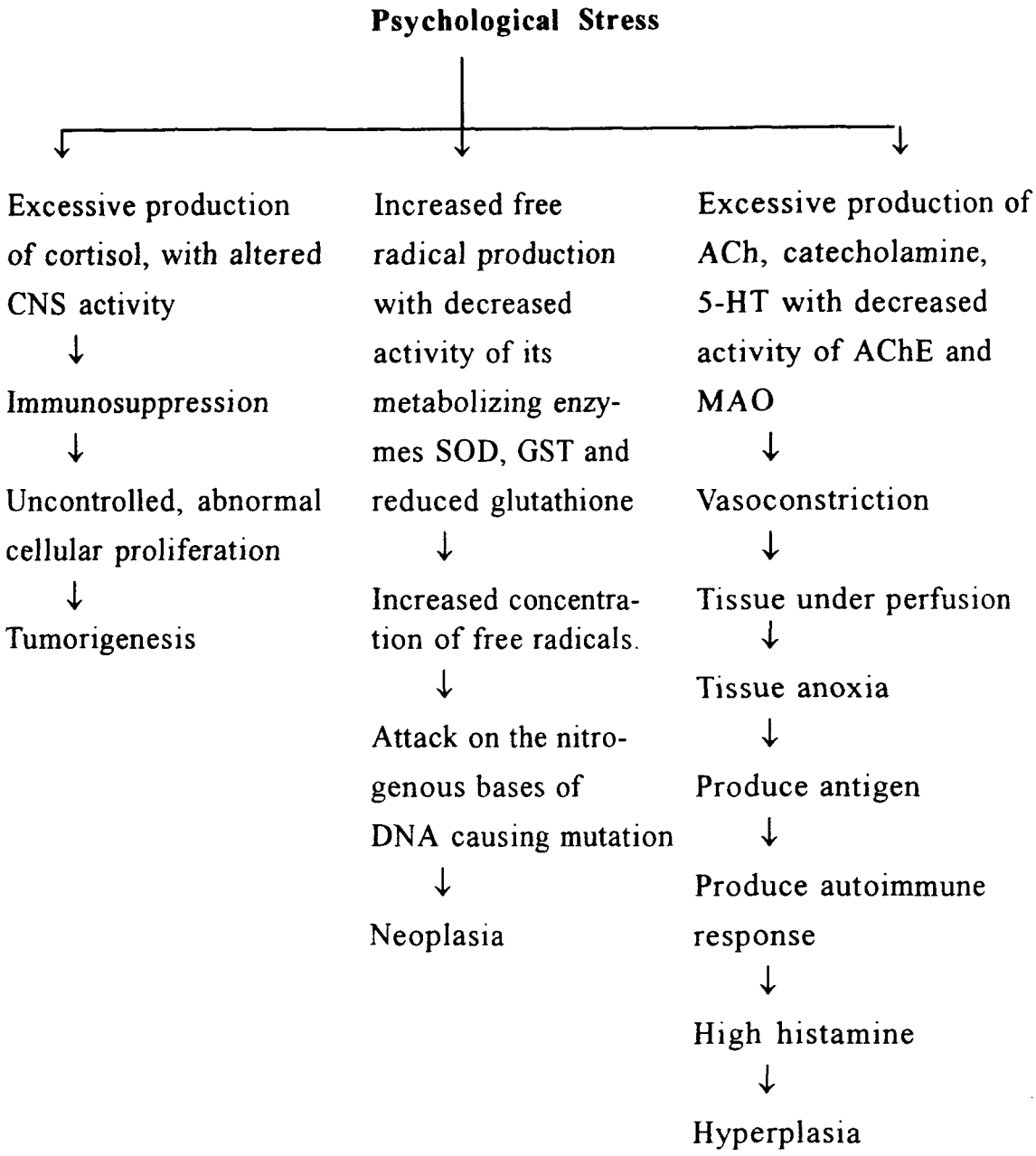
The Circulating activities of AChE and MAO were significantly decreased, the levels of cortisol, GOT, GPT and LDH were significantly elevated in both pre or post stress treated DMBA infused rats as compared to either stressed/or DMBA alone treated rats.

The brain, liver, kidney, heart and spleen levels of AChE, GST, SOD and reduced glutathione levels were decreased in pre and post stress treated DMBA infused rats, with a similar pattern as observed in DMBA alone or stress alone treated rats, but, the decrease in these parameters observed here was more significant. Similar to the significant effect of stress seen on the alterations of biochemical parameters the apparent physical state of the rats also worsened, because of which the post-stress DMBA infused rats were sacrificed on 100th day and pre stress DMBA infused rats on 110th day of commencement of the experiment. This clearly indicates that chronic restraint stress exposure (considered as both physical and psychological stress) has profound effect on initiation and progression of DMBA induced carcinogenesis by altering the levels of both neurohumors and free radical metabolizing enzymes. This is further confirmed by histopathological examination of the liver tissues of post stress DMBA infused rats, which showed that the disease was fairly advanced and metastasis occured to liver cells.

The restraint stress is found to facilitate DMBA induced mammary tumor by releasing beta-endorphin and prolactin too and naltrexone, an optiate analogue showed a beneficial effect by opposing the effects of beta-endorphin on prolactin release in the stressed animals (Tejwani *et al.*, 1991). β-endorphin binds to monocytes, lymphocytes and granulocytes and appears to have variable effects on NK cells (Chiapelli, 1991). Several studies have shown the role of psychological stress in initiation,

promotion or progression of cancer (William, 1988; Fox, 1981). Some studies showed that psychological factors play an important role in promotion and progression of cancer than its initiation (Fox, 1981; Temoshok, 1984; William, 1988). While the present study suggests that stress plays an important role not only in initiation but also in progression of DMBA induced carcinogenesis. Thus, stress through alteration of neurohumoral and free radical metabolizing enzymes may facilitate the initiation or progression of DMBA induced carcinogenesis as discussed. Moreover, the expression of the stress inducible 72 KDa heat shock protein (HSP72) on the surface of human tumor cells, but not on normal cells might imply clinical application as a means to target a stress-inducible, tumor-specific immune response (Mutthoff *et al.*, 1995). The inhibition of mammary tumor cell attachment to immobilized laminin or fibronectin by exposure to oxidative stress is shown to enhance experimental metastatic potential (Kundu *et al.*, 1995). Further, the enzyme generated (hypoxanthine and xanthine oxidase, which generates superoxide radical and H_2O_2) oxidative stress is found completely reversed by catalase (Kundu *et al.*, 1995). The decreased SOD, GST and reduced glutathione levels as observed in the stressed (pre and post) DMBA treated rats might have enhanced the damage. Surgical stress through increased glucocorticoid activity is reported to enhance metastasis in cancer patients (Kodama *et al.*, 1992). Similarly, a significantly enhanced level of cortisol is seen in stress treated cancerous rats here. Moreover, it has been demonstrated

that induction of stress response leads to genetic instability (Boesen *et al.*, 1992). Brain also contributes to stress induced suppression of cellular immune function (Ader and Cohen, 1991). A pathway of communication between the central nervous system and immune system has already been established (Felten *et al.*, 1987). In light of all these studies on stress and the alterations of neurotransmitters and free radical metabolizing enzymes as observed in the present study, it is presumed that these patients or experimental rats have raised ACh, catecholamine, histamine, 5-HT, cortisol, free radical levels (reactive oxygen species) with decreased activities of their metabolizing or scavenging enzymes and a decreased immune response from the very beginning in response to stress and these factors may contribute casually to the development of cancer by virtue of special risk factors or once the cancer has been developed or induced by chemical carcinogens, further exposure to stress becomes lethal and may be responsible for spread of cancer to other organs, as metastasis of cancer has been observed, generally, after surgical trauma in cancer patients (Kodama, 1992). Thus, the management of stress is important even for the treatment of cancer. In view of the known biochemical components in stress response, if at all stress contributes to the development of cancer, its mode of operation may be as follows :



Stress and biochemical parameters :

Stress is the nonspecific response of the organism to any demand made upon it. It operates through a system of regulatory measures associated with the mobilization of energy sources, enabling the organism to meet the adverse situations. It is accomplished by a complex of neuronal, endocrinal, circulatory and metabolic processes.

It has been shown that after any stress or trauma the first and the earliest change that takes place in the body is the alteration in the neurohumoral content because of central nervous system activation (Hass and George, 1988). Integrative physiologic models have implicated CNS in stress induced suppression of immune response (Keller *et al.*, 1988; Ader and Cohen, 1991).

It has been postulated by many workers that there is excessive production of all neurohumors of the body after trauma (Rai, 1976; Prasa, 1978 and Giraldi, 1994). In emotional stress far more pronounced changes are seen, catechoamines and cortisol are released in excess quantity from adrenal medulla and Cortex (Henry, 1980). Stress has been shown to bring about an increase in the activity of DBH and PNMT (Sharma, 1978 and Sarkar, 1978) and a decrease in the activity of MAO and AChE (Litvak, 1969 and Sharma, 1978). This has been confirmed in the present study as well. The decreased AChE activity will slow down the breakdown of ACh and thus may help in enhancing cholinergic stimulation.

In order to maintain the stability of a living organism, it is necessary to reach a balance between the oxidation actions and the antioxidant defence ie. anti-FRS. Enhanced free radical production with lipid peroxidation has been reported during stress (Clemens, 1991). The decreased activity of GST and SOD with a decreased level of reduced glutathione as observed in the present study may further aggravate this situation. The free radicals induce increased membrane permeability through membrane

lipid peroxidation, protein oxidation and histamine release from mast cells (Clemens, 1991). Thus, causing increased hemolysis of erythrocytes at higher concentrations of saline than the normal erythrocytes as seen in the present study.

Thus, cortisol, MAO, AChE alongwith free radical scavenging system GST, SOD and glutathione, play a decisive role in initiation, promotion and progression of stress induced carcinogenesis.

The effect of indigenous drugs on DMBA induced carcinogenesis :

The decrease in the circulating levels of cortisol, LDH, GOT, GPT and increase in the activities of MAO and AChE towards normal values were observed after treatment of the DMBA infused rats with indigenous drugs. The tissue activities of GST, SOD and AChE, the levels of reduced glutathione were elevated in the drug treated DMBA infused rats as compared to untreated DMBA infused rats. The efficacy of the drugs was evaluated in terms of the extent of reversion of these parameters from untreated DMBA infused towards normal values. Thus, the post treatment with garlic was found maximally effective in preventing DMBA carcinogenesis, while pre-treatment with salvia showed minimal effect. The pre-garlic and post salvia treatment showed nearly the same effect in prevention of DMBA induced carcinogenesis in rats. Uptil now the efficacy of various drugs or compounds on inhibition or promotion of experimental cancer was evaluated in terms of number of observed tumors per animal

and the rate of tumor development, which have their own limitations (Kokoska *et al.*, 1993), but in the present study the effect of the indigenous drugs on DMBA induced mammary carcinogensis is evaluated in terms of altered biochemical parameters in cancer.

The preventive or curative effects of garlic reside in its water soluble organo-sulfur components (s-allyl-cysteine, s-ethyl-cysteine and s-propyl cysteine) and oil soluble organosulfur components (diallyl sulfide, diallyl disulfide and diallyl trisulfide) which are reported to markedly inhibit tumor growth (Amagase and Milner, 1993). The action of these compounds seems to be mediated through alteration of neurohumoral and free radical metabolizing enzymes and thereby changing the levels of neurohumors and free radicals. The elevated levels of MAO and AChE after drug treatment might have helped in enhanced degradation of catecholamines, 5-HT and acetylcholine, thereby, reducing their enhanced levels seen in cancer (Litvak, 1969 and Sharma, 1978).

The increase in glutathione level by garlic treatment could be due to the selenium present in garlic (Sundaram, 1996). Glutathione treatment (Shklar *et al.*, 1993) or increased level of glutathione due to selenium treatment (Schwartz and Shklar, 1996) is shown to reduce tumor burden. Selenium is also shown to modulate the lipid peroxidation in DMBA induced-tumor rats (Chidombaram and Baradrajan, 1995), which may also account for the effect of garlic on RBC membrane fragility in the presence of saline.

Moreover, the antioxidants are also shown to enhance the immune response in tumor (Schwartz *et al.*, 1990). Similar to our findings, the levels of glutathione and GST have been shown enhanced following consumption of garlic or sulfur compounds (Sumiyosni and Wargovich, 1990). Selenium probably decreased the binding of DMBA metabolites to mammary cell DNA, and thus reduces the incidence of chemically induced tumors (Liu *et al.*, 1991). Similarly, garlic too because of its selenium content reduces the binding of DMBA metabolites to mammary cell DNA (Liu *et al.*, 1992; Amagase and Milner, 1993). The dietary selenium is also reported to enhance the efficacy of garlic as an anti carcinogenic agent (Ip *et al.*, 1992). The enhanced GST and SOD levels after treatment with garlic extracts may help in management of H_2O_2 produced by macrocphages in responses to altered immune system. The level of LDH, the tumor marker is also decreased towards normal values after garlic treatment, showing a decreased lactate metabolism. Thus, by garlic treatment both the immune (probably also through decrease in cortisol level) and free radical metabolizing responses are enhanced and the condition of rats is improved, which is also evident by a decrease in the levels of GST and LDH. At the end of the experiment all the post garlic treated animals were still alive, the average size of palpable mammary tumor was small and improvement was observed in terms of weight of the animals and stability of RBC membrances too. Thus, it can be concluded that garlic may prevent both initiation and promotion of cancer, but its preventive effect on promotion of DMBA carcinogenesis is more marked.

The indigenous drug salvia, is found to contain flavonoids and phenolic compounds (Andrew, 1996). Salvia and the essential oils extracted from it have been shown to exhibit antioxidant potential (Sastri, 1956). The phenols and flavonoids have a wide spectrum of pharmacological properties (Bertz *et al.*, 1977), and have been reported to inhibit carcinogenesis and mutagenesis in experimental animals (Ames, 1983 and VanHoff *et al.*, 1984). This raised our interest to evaluate its possible efficacy in inhibiting the initiation and promotion of cancer, possibly through alteration of free radical metabolism. The polyhydric phenols present in salvia might have inhibited cancer promotion due to the induction of cytochrome P-450 and other metabolic enzymes (Boyd *et al.*, 1982). The increased activity of SOD, GST and reduced glutathione pool is observed in post salvia treated DMBA infused rats, which may indicate decrease in free radical content. Thus, an improvement in the apparent conditions of rats was seen which correlated well with the weight gain and erythrocyte hemolysis.

Salvia has both estrogenic and nervous system stimulating effects (Andrew, 1996) which may account for slightly increased MAO and AChE activities as observed in salvia treated DMBA infused rats. Thus, salvia treatment might have altered the neurohumoral levels too, alongwith enhancement of free radical metabolizing enzymes. The decrease in the levels of cortisol after salvia treatment may indicate a reduction in immunosuppression.

Salvia showed a better preventive effect on promotion than initiation of DMBA induced carcinogenesis. The reduced efficacy of slavia in comparsion to garlic could be due to its esterogen content as glucocorticoid supplementation is found to cause depression and psychiatric disorders (Norman, 1998).

Stability of RBC membrane under various conditions

The maximum hemolysis of the RBC membranes from restraint stress treated and DMBA infused rats was at 0.4% saline and for DMBA infused pre and post restraint stress treated rats at 0.3 and 0.6% saline in phosphate buffer (PBS) respectively, while the maximum hemolysis of RBC from control rats was seen at 0.1% PBS. Thus, the erythrocytes from DMBA infused rats with post stress treatment showed maximum hemolysis at highest saline concentration (near normal saline) as compared to RBCs from only restraint stressed / or only DMBA infused / or DMBA infused pre-stress treated rats.

The RBC from pre-garlic and pre and post salvia treated DMBA infused rats showed maximum hemolysis at 0.2%, while post garlic treatement reverted it towards normal value.

Irrespective of the treatment given to the rats, the minimum RBC hemolysis observed was at 0.9% PBS, which is a normal phenomena.

Erythrocytes have life span of about 120 days, smith (1995) reported that exercise, cycling, running and swimming have been shown to cause

RBC membrane damage. Moreover, heat shock of 42°C is found to increase osmotic fragility of erythrocytes than seen at 37°C (Kogawa *et al.*, 1995). Similarly, the restraint stress showed an effect on the fragility of RBC membrane in the presence of various concentrations of saline. Immobilization stress induces generation of reactive oxygen species and decreases the endogenous antioxidant defenses (Liu *et al.*, 1994), which is also seen in the present study. Zima *et al.* (1996) reported that reactive oxygen species and other free radicals in the erythrocytes can cause reduced activities of SOD and GST with a depletion of reduced glutathione in restraint stress which may be the cause for the change in the stability of erythrocytes the change in the stability of erythrocyte membranes possibly because of free radical induced damage to RBC. Erythrocyte hemolysis is dependent upon cell volume, surface area and functional integrity of cell membranes. Incubation of RBC with lactic acid for one hour at 37°C increased the osmotic fragility of erythrocytes (Kogawa, 1995).

Reactive oxygen species and other radicals can cause erythrocyte hemolysis, which is one of the pathogenic mechanisms of anemia in cancer patients (Zima *et al.*, 1996). In the cancer patients and in experimental carcinogenesis, the activities of superoxide dismutase and glutathione-S-transferase are found significantly lowered which may enhance the free radical induced damage to the cells including erythrocytes, this may account for the susceptibility of RBC membranes to hemolysis at higher

percent of saline concentrations than normal. Leakage of LDH from erythrocytes has been reported in all cases of cancer (Singh, 1981 and Ts'ao *et al.*, 1996), which may be due to the membrane peroxidation caused by free radicals. Thus, the cell damage precipitated by protease, elastase and Triton probably involves hydrolysis of protein and phospholipids in the cell membrane, leading to an enhanced leakage of intercellular protein such as LDH (Ts'ao *et al.*, 1996). The level of LDH is seen enhanced is both experimental carcinogenesis and clinical cancer patients.

Both in stress and neoplastic diseases, the free radicals cause enhanced membrane peroxidation of the blood than seen in the normal subjects (Clemens, 1991). It has been observed that free radicals induce increased membrane permeability through membrane lipid peroxidation, protein oxidation and histamine release from mast cells and LDH and AChE release from RBC (Rovere *et al.*, 1995). Moreover, they also cause formation of oxyhemoglobin and hemichromes (Rovere *et al.*, 1995). Thus, the damage caused to RBC membranes may be responsible for the change of hemolysis and release of hemoglobin observed from minimum concentration of saline (0.1%) at which normal RBC have shown maximum hemolysis towards a higher concentration of saline (0.3, 0.4 and 0.6% PBS) at which DMBA induced cancerous, restraint stress treated and DMBA infused with stress treated RBCs showed maximum hemolysis. Thus, the results of erythrocytes hemolysis confirm the previous findings on free radical lipoperoxidation resulting in the damage of RBC

membranes hemolysis and anemia seen in tumoral diseases (Clemens, 1991).

From both the RBC hemolytic and biochemical studies, it is evident that the exposure of rats to stress after DMBA induced carcinogenesis had maximum deleterious effect than the pre stress treatment. Thus, stress may be responsible for the enhancement of the progression of carcinogenesis.

Change in weight of DMBA infused rats with stress or indigenous drugs treatment :

A gradual increase in the weights of normal rats was observed after commencement of the experiment till the end as expected. A slight weight gain or loss in weight was observed for the rats which were treated with DMBA (alone) or DMBA infused with pre and post stress treatment. The weight gain of post stress DMBA infused rats was maximally effected. The treatment of DMBA infused rats with the indigenous drugs showed a slight improvement in weight gain. The post garlic treatment was found to be maximally effective in controlling the weight loss caused due to DMBA carcinogenesis.

The increase in weight with age is a normal phenomenon of growth and development. The weight loss or a little weight gain with age as has been observed in DMBA infused cancerous and pre and post stress treated DMBA infused rats is quite obvious as anorexia and catchexia are the hallmark of cancer. The feeding control mechanism is found increasingly

impaired with the advancing tumor (Morrison, 1976). The hypothalamus in the brain is responsible for regulation of hunger and satiety. Moreover, neurohumors like 5-HT and epinephrine, are also responsible for hunger (Change *et al.*, 1983). This regulation primarly depends upon the interaction between two areas in the hypothalamus; the lateral hunger centre and the ventrolateral satiety centre. The lateral center is also involved in the neural regulation of the appetite. Thus, the stress of the disease might be responsible for both loss/or little gain in weight and the deteriorating conditions of rats. The post stress exposure on DMBA infused carcinogenesis showed maximum effect on the apparent condition of rats and the alteration of biochemical parameters. Similarly, the development of hepatic cancer observed in the present study may be due to the restrait stress exposure after DMBA infusion.

The weight loss seen due to DMBA induced carcinogenesis was reduced by drug treatments. Both in terms of weight gain and the reversion of biochemical parameters, the post garlic treatment had a better effect on prevention of DMBA induced carcinogenesis in rats than either pre garlic or pre/post salvia treatment.

Summary

1. Stress plays an important role in causing and prolonging mental and physical illness. Exposure to any type of stress leads to neuroendocrine, endocrine and metabolic changes in the organism. Under normal situations, all these responses act as defense mechanism and help the organism to face the stressful situations more effectively. However, in case the organism fails to adapt to these conditions, the above mentioned response may last long and functional disability of one or more organs may develop and may precipitate in one or the other disorders commonly termed as stress disorders.

2. The malignant transformation of a normal cell and its further progression in the human body is a polygenic phenomena. The stress and strain of the competitive life style may be one of the predisposing factors for cancer.

3. The present study has been divided into two parts, clinical and experimental. In the clinical study, the circulating levels of AChE, MAO, cortisol, LDH, GOT and GPT were estimated in psychiatric (Anxiety neurosis), cancer breast and cancer liver patients. These have been compared with the values obtained from normal controls of both the sexes. A follow-up study was also carried out in all these patients after treatment in terms of the above mentioned parameters.

4. In the experimental part, the circulating levels of AChE, MAO, cortisol, LDH, GOT and GPT and tissue levels of SOD, GST, AChE and reduced glutathione (total, free and protein bound) were estimated in restraint stressed (immobilized), DMBA induced mammary cancer, DMBA induced cancer with pre and post restraint stress, DMBA induced cancer with pre and post garlic/salvia treated rats. These have been compared with values from their respective normal controls/restraint stressed/DMBA induced cancerous rats.

5. The activities of plasma MAO, RBC AChE and osmotic fragility and levels of serum LDH, GOT and GPT, tissues AChE, SOD, GST and reduced glutathione (total, free and protein bound) were determined by spectrophotometric procedures, while the levels of plasma cortisol was measured by use of spectrofluorometer.

6. In normal humans there is no statistically significant sex-based difference in the levels of AChE, MAO, cortisol, LDH, GOT and GPT. The observed levels of AChE, MAO and cortisol were slightly higher in females, while the levels of LDH, GOT and GPT were higher in males but the difference was statistically insignificant.

7. In the patients of anxiety neurosis, the circulating levels of cortisol,GOT, GPT and LDH were significantly raised and the activities of RBC AChE and plasma MAO were significantly decreased. The biochemical parameters reverted towards their corresponding normal values after one

month of chemotherapy in anxiety neurotic patients and were less significantly altered from normals. A slight but insignificant increase in the levels of SGOT and SGPT were observed after treatment.

8. Circulating cortisol, GOT, GPT and LDH levels were significantly raised while RBC AChE and plasma MAO activities were decreased in the patients of breast cancer. After one month of treatment (surgery, chemotherapy and radiotherapy), these levels reverted towards control values except the levels of GOT and GPT which showed a slight increase.

9. In the patients of cancer liver the circulating levels of cortisol, GOT, GPT and LDH were raised significantly as compared to controls whereas the activities of RBC AChE and plasma MAO were significantly decreased. After one month of therapy all these parameters remained significantly altered, the levels reverted towards control values but still remained significantly altered. There was a slight increase in the levels of SGOT and SGPT after treatment.

10. For the experimental part female Spraque-Dawley rats were selected and the study was performed as discussed below.

11. The circulating activities of AChE, MAO, levels of cortisol, GOT, GPT, LDH and RBC membrane osmotic fraglility were estimated in normal female rats. The activities of AChE, SOD and GST, levels of reduced glutathione (total, free and protein bound) were estimated in various tissues (brain, liver, kidney, heart and spleen) of control rats. In comparision to

other tissues, the brain tissues had maximum activity of AChE while kidney tissues showed minimum activity. Maximum activity of GST was in the liver, while minimum was recorded in the heart tissues. The brain and spleen tissues had minimum SOD activity while liver had maximum as compared to other tissues.

12. For the restraint stress studies the female rats were immobilized for different time intervals as 6, 12, 18, 24 and 30 hours. The above mentioned biochemical parameters were assayed after sacrificing these rats. The alterations seen in the activities of RBC AChE, plasma MAO and the levels of cortisol, LDH, GOT and GPT in the circulation were minimum at 6 and 12 hours, while a gradual alterations were recorded at 18 hours, a maximum change was observed at 24 hours. At 30 hours, a slight reversion towards normal was seen in the activities of AChE, MAO, the levels of cortisol and LDH. There was slight increase in the activities of GOT and GPT at 30 hours of restraint stress treatment.

13. As the changes observed in the above mentioned biochemical parameters were maximum at 24 hours of stress, thus, in further studies, the rats were exposed to 24 hours of stress to see it's effect on DMBA carcinogenesis. In these 24 hours stress treated rats, the activities of RBC AChE, plasma MAO were significantly decreased, while circulating levels of cortisol, GPT and LDH were significantly elevated. A less significant increase was seen in the serum GOT levels. The activities of AChE, GST and.

SOD, the levels of total, free and protein bound GSH were significantly decreased in the tissues (heart, liver, kidney, spleen and brain) of restraint stressed rats as compared to values obtained from their respective normal tissues.

14. Cancer was induced by a single oral dose of DMBA (30 mg/kg body weight). The effect of pre and post restraint stress on DMBA infused rats was also studied.

15. The circulating activities of AChE and MAO were significantly decreased, while the levels of cortisol, GOT, GPT and LDH were significantly elevated in DMBA treated rats. The activities of AChE, SOD, GST and the levels of reduced glutathione (total, free and protein bound) were significantly decreased in these rats. While a further alteration was recorded in the levels of the above biochemical parameters in DMBA infused rats with pre and post-stress treatment. The post stress treatment to the DMBA infused rats caused maximum alterations, severe than pre-stress treatment as compared to the values obtained from their respective normal rats.

16. Irrespective of the treatment given to the rats the minimum RBC hemolysis observed was at 0.9% phosphate buffered saline (PBS) pH 7.4, while the maximum was as follows: For normal 0.1%, DMBA treated and restraint stressed rats 0.4%, pre-stress DMBA infused 0.3% and post stress DMBA infused 0.6% saline in 0.1 M phosphate buffer pH 7.4.

17. The maximum fragility of RBC membranes towards saline concentration was reverted to the control values after drug treatment. The pre-garlic treated DMBA infused rats showed maximum hemolysis of RBC at 0.2%. While post garlic treatment reverted it towards normal i.e. 0.1%. The RBC of pre and post salvia treated DMBA infused rats had maximum hemolysis at 0.2% phsophate buffered saline.

18. A gradual increase in the weights of normal rats was observed after commencement of the experiments till the end, while in DMBA treated or pre-and post stress treated rats with DMBA infusion either the weight was lost or there was a slight increase in weight. The weight of post stress DMBA treated rats was maximally effected. The treatment of DMBA infused rats with the indigenous drugs showed a slight improvement in weight. The post garlic treatment was found to be maximally effective in controling the weight loss.

19. In the animals pre-treated with garlic and salvia before induction of cancer with the DMBA, the changes recorded above were less marked than in the untreated animals. The pre-treatment with garlic was more effective in preventing the changes in the above biochemical parameters than the pre-treatment with salvia. Eventhough the cancer was developed in these drug treated rats but their conditions were better than those of untreated DMBA infused cancerous rats in terms of either the weight or the above mentioned biochemical parameters.

20. The post-treatment of the rats with these drugs (garlic and salvia) after DMBA infusion brought about changes in all the parameters to bring these a little closer to the values in control animals.With both the drugs (garlic and salvia), Post-treatment was more effective than the pre-treatment. Thus, both drugs can be said to have a better preventive effect on promotion than initiation of DMBA induced cancer so far as the biochemical parameters are concerned.

21. The post stress treatment on DMBA infused cancerous rats had more severe effect than pre-stress treatment in terms of changes in weight or biochemical parameters.

22. The effectiveness of garlic and salvia on DMBA carcinogenesis in terms of weight or biochemical parameters can be summarized as : post treatment with garlic > pre-treatment with garlic ≥ post treatment with salvia > pre treatment with salvia.

Conclusion

The circulating levels of cortisol, LDH, GOT and GPT were increased, while the activities of AChE and MAO were decreased in the patients of anxiety neurosis, cancer breast and cancer liver. The change in the above parameters were more marked in cancer patients than the anxiety neurosis.

The alterations of these parameters have a direct correlation with the severity of the disease. Since the number of patients investigated were less, it is felt that further work along these lines would be necessary to have a large statistical sample. These studies may lead to the development of a diagnostic tool to monitor the progress or to give an early information about cancer.

A good correlation exists between the experimental and clinical conditions of stress disorders, restraint stress and DMBA induced carcinogenesis as regard to neurohumors and free radical metabolizing enzymes.

In experimental rats, the activities of AChE, SOD and GST, and the levels of reduced glutathione (total, free and protein bound) were decreased in tissues (brain, heart, liver, kidney and spleen) of restraint stress, only DMBA infused and DMBA infused with pre and post stress treated rats. Though the pattern of changes in the above biochemical parameters were the same under different conditions, the severity of change was maximum in DMBA infused rats with post

stress treatement than in restraint stressed (only), DMBA infused (only) or DMBA infused rats with pre-stress treatment, which indicated the effect of post stress on DMBA induced carcinogenesis, resulting into fast deterioration of apparent health and tumor incidence in cancer. The post stress treatment resulted in metastasis of cancer to liver cells too.

The decreased activities of AChE may enhance the levels of ACh in patients and experimental rats which may further indicate an enhanced activity of parasympathetic nervous system. The severity of the disease increases with decrease in AChE levels. The significantly enhanced levels of plasma cortisol and decreased activity of plasma MAO may suggest an activation of the sympathetic-hypothalamic-pituitary adrenocortical axis (Henry, 1977). The significant increase in the cortisol levels may be due to the stress of the disease itself. The enhanced levels of cortisol as observed in patients and experimental animals may be responsible for immunosuppression in these cases.

The plasma levels of LDH, GOT and GPT were found increased in both clinical and experimental carcinogenesis and stress. These levels have already been reported to act as tumor markers (Vinitha *et al.*, 1995). The present study correlated well with the previous findings. The increase in these parameters was more marked in post

DMBA infused rats in comparision to other experimental conditions, showing the severity or metastais of cancer thereof, to the liver cells. Thus, there was a direct correlation between the rise in these parameters and severity of the disease.

The decrease in the tissue activities of SOD and GST and the levels of reduced glutathione may be due to stress and cancer induced excessive generation/accumulation of free radicals which caused more damage in the system.

The minimum RBC membrane hemolysis was seen at 0.9% saline in phosphate buffer irrespective of the treatment given to the rats, while there was a difference in maximum hemolysis.

The maximum hemolysis of normal erythrocytes was at 0.1% PBS, while the erythrocytes from restraint stressed, DMBA infused and DMBA infused with pre and post stress treated rats showed maximum hemolysis at higher saline concentrations reflecting a cahnge in the RBC membrane structure and permeability, which may be due to free radical induced membrane damage and lipid peroxidation leading to LDH leakage from RBC to plasma.

In patients of anxiety neurosis and cancer breast, the above mentioned parameters were reverted significantly towards normals after the required course of treatment, while this reversion towards normal was lesser in cancer liver. The levels of serum GOT and GPT

were slightly increased after treatment which may be due to the side effects of treatment given to the patients. Similar observations were seen in DMBA infused rats with indigenous drug treatments, the post garlic treatment was most effective in preventing DMBA induced carcinogenesis as far as different biochemical parameters, RBC hemolysis and body weights were concerned, while the pre-saliva treatment showed the least effect. The effectiveness of these drugs were in the following orders : post garlic treatment > pre-garlic treatment $\geq$ post salvia treatment > pre salvia treatment. Thus garlic showed preventive effect on initiation and promotion of DMBA induced cancer, while salvia had preventive effect only on the promotion of DMBA induced carcinogenesis.

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LIST OF PUBLICATION / PRESENTATION

1. ***Moayad Khataibeh*** and Naheed Banu: Role of restraint stress on intiation and promotion of DMBA induced cancer in rats. At 17th Annual Convention of the Indian Association for Cancer Research and National Symposium on Breast Cancer, Calcutta-India, Jan. 12th- 24th 1998.
2. Naheed Banu and ***Moayad Khataibeh***: "Modulation of DMBA induced cancer by Garlic extract in terms of Biochemical parameters". At 17th Annual Convention of the Indian Association for Cancer Research and National Symposiun on Breast Cancer, Calcutta-India, Jan. 12th-24th, 1998.
3. ***Moayad Khataibeh*** and Naheed Banu: "Role of stress in cancer : Through neurotransmitters and free radical metabolizing enzymes". At 14th Asia Pacific Cancer Confrence and 4th HongKong International Cancer Congress, Hongkong, Nov. 16th-19th, 1997.
4. ***Moayad Khataibeh***, Sandeep Sinha and Naheed Banu. "Biochemical alterations in stress and cancer", Chemical and Environmental Research, 5 (1-4), 169-173, 1996.
5. ***Moayad Khataibeh*** and Naheed Banu: "Biochemical alterations in stress and breast cancer". At the Second International Conference on Breast Cancer, Jinan China, Oct. 14th-16th 1996.
6. ***Moayad Khataibeh***, Sandeep Sinha and Naheed Banu. "Alterations in stress and cancer". At International Conference on Molecular Association- ICMA, Aligarh-India, March18th-22nd, 1996.
7. Naheed Banu, Sandeep Sinha and ***Moayad Khataibeh***. "Neurohumors, stress and cancer". At 12th Asia Pacific Cancer Conference, Singapore, Oct.17th-20th,1995.
8. ***Moayad Khataibeh***, Sandeep Sinha and Naheed Banu." Cancer: Role of stress through Neurohumors". At 16th Annual Meeeting of Society of Biological Chemist of India, Lucknow, Oct.6th-8th,1995.